Chemical Investigation of Three Plants Used in Cameroonian Traditional Medicine: *Maesopsis eminii* (Rhamnaceae), *Autranella congolensis* (Sapotaceae) and *Pentadesma grandifolia* (Guttiferae)

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by

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Contents

Glossary

Api	β-D-Apicose
APT	Attached Proton Test
Ara	β-L-Arabinose
br	Broad (NMR, IR)
$CDCl_3$	Deuterated chloroform
CD_3OD	Deuterated methanol
C_5D_5N	Deuterated pyridine
$(CD_3)_2CO$	Deuterated acetone
$CHCl_3$	Chloroform
CH_2Cl_2	Dichloromethane
CI	Chemical ionisation
COSY	Correlation Spectroscopy
d	Doublet (NMR)
δ	Chemical shift (NMR)
DEPT	Distortionless enhancement by polarisation transfer
DMSO	Dimethyl sulfoxide
DMSO-D_6	Deuterated Dimethyl sulphoxide
D_2O	Deuterated water
EI	Electronic impact
EtOAc	Ethyl acetate
EtOH	Ethanol
ESI	Electrospray ionisation
FAB	Fast atomic bombardment
FT	Fourier transform
Gal	β-D-Galactose
Glc	β-D-Glucose
HIV	Human immunodeficiency virus
HMBC	Heteronuclear multiple bond correlation
HMQC	Heteronuclear multiple quantum coherence
HPLC	High Performance Liquid Chromatography
HRMS	High Resolution Mass Spectrometry
IC_{50}	50% inhibitory capacity

Int.	Intensity
IR	Infrared
J	Coupling constant
m	Multiplet (NMR)
Me	Methyl
MeOH	Methanol
min	Minute
mp	Melting point
MS	Mass spectrometry
NMR	Nuclear magnetic resonance
PE	Petrol ether
ppm	Parts per million
PTLC	Preparative thin layer chromatography
R_f	Retention factor
Rhm	β-L-Rhamnose
s	Singlet (NMR)
t	Triplet (NMR)
TLC	Thin Layer Chromatography
UV	Ultraviolet
Xyl	β-D-Xylose

Summary

The work we carried out for the account of this thesis concerned the chemical investigation of three plants used in Cameroonian traditional medicine, *Maesopsis eminii* (Rhamnaceae), *Autranella congolensis* (Sapotaceae) and *Pentadesma grandifolia* (Guttiferae).

13 compounds were isolated from the bark of *Maesopsis eminii* (Engl.). Two pentacyclic triterpenes, 1α,3β-dihydroxybauer-7-en-28-oic acid (**85**) and 3β-dihydroxybauer-7-en-28-oic acid (**86**) of which (**85**) is reported here for the first time, together with four steroids labelled **91**, **92**, **24** and **25,** and seven phenolic compounds **87-90**, **93**, **9**, **97**+**98**, were isolated from this plant. **93** was derivatized to its acetate derivative labelled **94**. Triterpenes were isolated for the first time from this plant and the new compound **85** displays moderate antibacterial activity against Gram-positive bacteria such as *bacillus subtilis ATCC* while compound **88** displays good anti-inflammatory activity (IC_{50} 9.5 μM) by inhibiting highly the 3α-hydroxysteroid dehydrogenase.

Six known compounds have been isolated from the bark of the plant *Autranella congolensis* (De Wild.) A. Chev., taraxerol (**99**), taraxerone (**100**), a mixture of 3β-docosanoyltaraxer-14-ene (n = 20), 3β-tetracosanoyltaraxer-14-ene (n = 22), 3β-hexacosanoyltaraxer-14-ene (n = 24) (**101**), (+)-catechin (**97**), (24*R*)-stigmast-7,22(*E*)-dien-3α-ol (**26**) and 24-feruloyltetracosanoic acid (**102**).

Six known compounds have also been isolated from the root bark of *Pentadesma grandifolia* (E. G. Baker), a bicyclic triterpenoid (13*E*,17*E*)-polypoda-7,13,17,21-tetraen-3β-ol (**105**), lupeol (**23**) and four xanthones α-mangostin (**103**), rubraxanthone (**104**), garcinone E (**106**) and cowanin (**108**). Compounds **104** and **105** displayed good anti-inflammatory activity when tested against 3α-hydroxysteroid dehydrogenase. In addition, **104** selectively inhibits the cyclooxygenase-2 (COX-2).

Methods used for the isolation of compounds were mainly column chromatography, preparative TLC, and HPLC using solvents with differents polarities and selectivity.

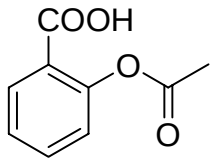
The structures of all compounds were elucidated by using modern spectroscopic techniques such as 1D and 2D-NMR (HH-COSY, HMBC, HMQC), HRMS and IR spectroscopy.

1 Introduction

Natural products are naturally derived metabolites and/or byproducts from microorganisms, plants, or animals (Baker et al., 2000). In the field of traditional medicine, natural products have been exploited for human use for thousands of years, and plants have been the main source of compounds used for medicine.

Traditional healers have from time immemorial exploited the therapeutic properties of plants in traditional medicine. They have always known that a particular plant treats a disease because it contains a bioactive component or some agents responsible for its power to fight the diseases which they treat and which may not be found in another plant. They however do not know the active components in the plant, the compound(s) that is/are responsible for its medicinal use. The Chinese represent today the largest users of traditional medicines, with over 5000 plants and plant products in their pharmacopeia (Bensky and Gamble, 1993).

Nowadays, traditional medicine has given rise to modern therapy. Natural products have played, and will continue to play, a key role in drug discovery and are therefore traditionally claimed as the cornerstones of drug discovery and development (Cragg et al., 1997; Grabley and Thiericke, 1999; Newman et al., 2000). In fact, many drugs on the market today were discovered from natural sources; one important example is the analgesic activity of aspirin (**1**), which is so far the world's best known and most universally used medicinal agent; it is related to salicin, and has its origin in the plant genera *Salix* spp. and *Populus* spp. Another example is the antibiotic activity of penicillin (**2**) discovered serendipitously in the laboratory from the fungus *Penicillium notatum*.



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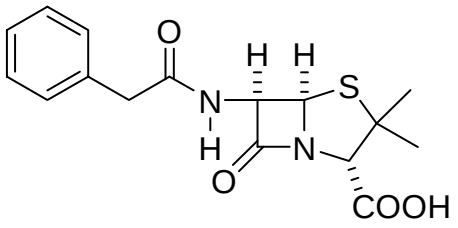

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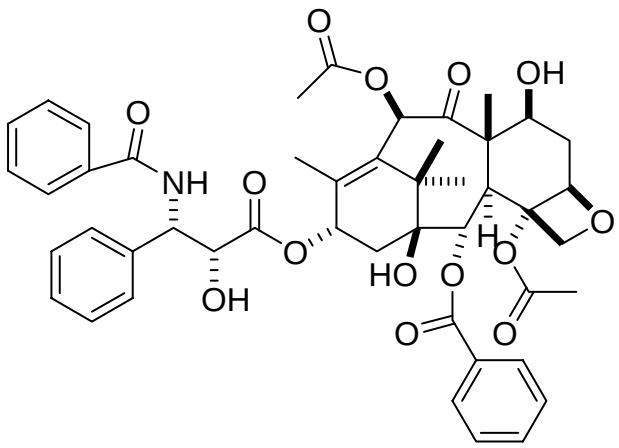
Strobel and coworkers demonstrated that natural product compounds have had, and will still have an immense impact on modern medicine: in fact, about 40% of prescription drugs are based on them (Strobel et al., 2004). Well over 50% of the new chemical products

registered by the FDA (Food and Drug Administration) in the time 1981-2002 as anticancer agents, antimigraine agents, and antihypertensive agents were natural products or derivatives thereof (Newman et al., 2003). Many other examples exist that illustrate the value and importance of natural products from plants and microorganisms in modern civilizations, and paclitaxel (taxol) (**3**), which was first isolated from the bark of the Pacific yew tree, *Taxus brevifolia* (Taxaceae), is the most recent example of an important natural product that has made an enormous impact on medicine (Wani et al., 1971; Bills et al., 2002). The WPS (White Point Systems) mentioned that synthetic and combinatorial methods would not yield such a complex structural type. A list of some drugs derived from plants, with their ethnomedical correlations and sources is given on Table 1.1.



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Unfortunately, some infectious microorganisms tend to develop resistance against some existing drugs. In addition to the resistance against some drugs, new diseases such as AIDS, Ebola and SARS are increasing, and therefore create a desperate need for new drugs (Strobel et al., 2004). In spite of the fact that much or if not most of what constitues modern drugs owes its presence directly or indirectly to chemicals originally found in plants, the vast majority of plants has not been assessed pharmacologically for potential medicinal value, even those that are currently being used for medicinal purposes by indigenous people (Akerele, 1992). The number of higher plant species (angiosperms and gymnosperms) on this planet is estimated at 250 000 (Fabricant and Farnsworth, 2001). Of these, only about 6% have been screened for biological activity, and a reported 15% have been evaluated phytochemically (Verpoorte, 2000). Thus, plants represent a largely untapped ressource. Beside the fact that they offer the local population immediate access to

safe and effective products for use in treatment of illness through self-medication, medicinal plants are valuable for modern medicine in four basic ways (Farnsworth and Soejarto, 1985; Plotkin, 1991):

(1) They are used as sources of direct therapeutic agents

(2) They serve as raw material base for the elaboration of more complex semi-synthetic chemical compounds

(3) The chemical structures derived from plant substances can be used as models for new synthetic compounds

(4) Finally, plants can be used as taxonomic markers for the discovery of new compounds.

Based on the long-term use of medicinal plants by humans (often hundreds or thousands of years), one might expect any bioactive compounds obtained from such plants to have low human toxicity. Obviously, some of the medicinal plants may be toxic but since there is no report on their chronic toxic effects, these plants will then continued to be used by the local populations.

As natural products gain increasing importance and attention from chemists and pharmacologists, their discovery from new sources will continue to be essential in order to provide novel lead compounds which the synthetic chemist can modify. This the major reason for embarking on research projects in the field of natural products. This work focuses on the isolation of potential drug candidates from plants collected in Cameroon. In order to establish a certain relationship between the therapeutic properties of plants and the natural products that plants may contain, three plants used in Cameroonian traditional medicine were investigated: *Maesopsis eminii* (Rhamnaceae), *Autranella congolensis* (Sapotaceae) and *Pentadesma grandifolia* (Guttiferae). The choice of the plants was based on the fact that their phytochemical studies were less reported in the literature for some, or completely absent for the others.

Table 1.1: Drugs discovery by ethnobotanical leads (Fabricant and Farnsworth, 2001)

Drug	Action or clinical use	Plant source
Acetyldigoxin	Cardiotonic	*Digitalis lanata*
Ajmaline	Circulatory disorders	*Rauwolfia serpentina*
Aspirin	Analgesic, anti-inflammatory	*Filipendula ulmaria*
Atropine	Anticholinergic	*Atropa belladonna*
Bergenin	Antitussive	*Ardisia japonica*
Bromelain	Anti-inflammatory, proteolytic agent	*Ananas comosus*
Caffeine	Stimulant	*Camellia sinensis*
(+)-Catechin	Haemostatic	*Potentilla fragaroides*
Cocaine	Local anaesthetic	*Erythoxylum coca*
Codeine	Analgesic, antitussive	*Papaver somniferum*
Colchicine	Antitumor agent, antigout	*Colchicum autumnale*
Danthron	Laxative	*Cassia spp.*
Deserpidine	Antihypertensive, tranquilizer	*Rauvolfia canescens*
Digotoxin	Cardiotonic	*Digitalis purpurea*
Digoxin	Cardiotonic	*Digitalis purpurea*
Emetine	Amoebicide, emetic	*Cephaelis ipeccuantha*
Ephedrine	sympathomimetic	*Ephedra sinica*
Etoposide	Antitumor agent	*Podophyllum peltatum*
Gitalin	Cardiotonic	*Digitalis purpurea*
Gossypol	Male contraceptive	*Gossypium spp.*
Hydrastine	Hemostatic, astringent	*Hydrastis canadensis*
Hyoscamine	Anticholinergic	*Hyoscamus niger*
Kawain	Tranquilizer	*Piper methysicum*
Khellin	Bronchodilator	*Ammi visnaga*
Lobeline	Smoking deterrent, respiratory stimulant	*Lobelia inflata*
Morphine	Analgesic	*Papaver somniferum*
Noscapine	Antitussive	*Papaver somniferum*
Ouabain	Cardiotonic	*Sthrophanthus gratus*
Papain	Proteolytic, mucolytic	*Carica papaya*
Physostigmine	Cholinesterase inhibitor	*Physostigma venenosum*
Picrotoxin	Analeptic	*Anamirta cocculus*
Pilocarpine	parasympathomimetic	*Pilocarpus jaborandi*
Protoveratrines A & B	Antihypertensive	*Veratrum album*
Pseudoephedrine	Sympathomimetic	*Ephedra sinica*
Quinine	Antimalarial	*Cinchona ledgeriana*
Quisqualic acid	Anthelmintic	*Quisqualis indica*
Rescinnamine	Antihypertensive, tranquilizer	*Rauwolfia serpentina*
Reserpine	Antihypertensive, tranquilizer	*Rauwolfia serpentina*
Rorifone	Antitussive	*Rorippa indica*
Rotenone	Piscicide	*Lonchocarpus nicou*
Salicin	Analgesic	*Salix alba*
Stevioside	Sweetener	*Stevia rebaudiana*
Teniposide	Antitumor agent	*Podophyllum peltatum*
Tetrahydropalmatine	Analgesic, sedative	*Corydalis ambigua*
Theobromine	Diuretic, bronchodilator	*Theobroma cacao*

Trichosanthin	Abortifacient	*Thymus vulgaris*
Tubocurarine	Skeletal muscle relaxant	*Chondodendron tomentosum*
Vincamine	Cerebral stimulant	*Vinca minor*
Xanthotoxin	Leukoderma, vitiligo	*Ammi majus*
Yohimbine	Aphrodisiac	*Pausinystalia yohimbe*
Yuanhuacine	Abortifacient	*Daphne genkwa*

To start the analysis, it is important to understand what are secondary metabolites and what is their importance.

1.1 Secondary metabolites from plants

Secondary metabolites are molecules that are not directly necessary for the growth and reproduction of a plant, but may serve some role in herbivore deterrence due to astringency or they may act as phytoalexins, killing bacteria that the plant recognizes as a threat. Secondary metabolites are often involved in key interactions between plants and their abiotic and biotic environments that influence them (Facchini et al., 2000). Plants produce secondary metabolites as defences against fungi, bacteria, insects and viruses. They also produce them as colourful pigments to attract insects for pollination. The exact nature of the role of many secondary metabolites is not known although they have been identified and extracted.

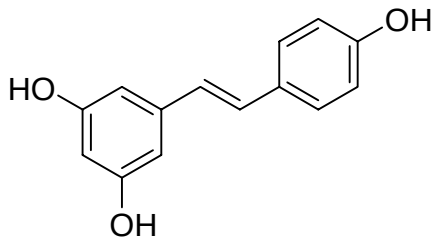
Secondary plant metabolites, currently exceeding 100.000 identified substances, belong to three major chemical classes: terpenoids (a group of lipids), phenolics (derived from carbohydrates) and alkaloids (derived from amino acids) (Edwards and Gatehouse, 1999).

1.1.1 Terpenoids

Terpenoids have been cited as the most diverse group of plants products known (Goodwin and Mercer, 1983). Many of these products have functions known to be essential to plant life (e.g. carotenoids, chlorophyll side-chain and some hormones) whereas the function of other terpenoids are unknown (Curry, 1987). Volatile monoterpernoids are the major components of essential oils and often function as floral odour glands (Goodwin and Mercer, 1983). Terpenoids are discussed in details in part 2.2 of this work.

1.1.2 Phenolics

Phenolic compounds are a huge and diverse group of aromatic compounds usually with hydroxyl groups. Phenol itself is the simplest member of the class, although it is not found in plants. Many phenolic compounds have three carbon side chains and are called "phenyl-propanoids". They include pigments, flavour compounds and tannins. They probably function in defense against herbivores and in regulation of auxin transport. Attraction of insects and birds also play an important role in seed dispercial and pollination (Goodwin and Mercer, 1983). The well-known phytoalexin, resveratrol (**4**), an anticancer agent is an example of a phenolic as are flavonoids and tannins which are found in tea, fruits and red wine and have many desirable health effects (Oomah, 2003).



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1.1.3 Alkaloids

Alkaloids, a major class of plant-derived secondary metabolites used medicinally has potent pharmacological effects in animals due to their ability to rapidly penetrate cell membranes (Wink, 1999). Nicotine, a commercially important alkaloid, is the most physiologically addictive drug used by humans. Caffeine, an alkaloid from coffee, tea and chocolate is a central nervous system stimulant and mild diuretic. The opium plant contains over 25 alkaloids with morphine being the most abundant and most potent painkiller. Vincristine and vinblastine, important alkaloids from periwinkle are strong antineoplastics used to treat Hodgkins disease and other lymphomas (Wink, 1999).

Secondary metabolites are sought after because they are known to exhibit numerous biological activities that promote positive health effects. These activities include antibacterial, anticancer, antifungal, and antioxidant that are utilized in the agricultural, food and pharmaceutical industries. As a consequence of these numerous applications, the world market for plant extracts and isolated secondary metabolites exceeds 10 billion US

dollars annually (Oomah, 2003). The pharmacological value of plant secondary metabolites is increasing due to constant discoveries of their potential roles in health care and as lead chemicals for new drug development (Wink, 1999).

2 Literature review

2.1 Previous study of the genera *Maesopsis*, *Autranella* and *Pentadesma*

2.1.1 Generalities on the Genus *Maesopsis*

M. eminii was introduced from the Bukoba regions in western Tanzania, to the Amani Botanic Gardens in the East Usambara (eastern Tanzania) in 1913 when a 1 ha forestry trial plot was set up. Large-scale forestry planting was undertaken during the 1960s and early 1970s. *M. eminii* is now dominant in secondary forests near the *M. eminii* plantation and is found in many natural forest treefall gaps (Binggeli and Hamilton, 1993).

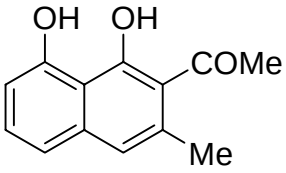
The genus *Maesopsis* belongs to the plant family Rhamnaceae. This genus is monospecific (Hallé, 1970) and is widely distributed throughout the African continent, especially in tropical region, from Liberia to Congo, Sudan, Uganda and East Africa. In Cameroon this plant is present in the Centre and Southern Provinces. Usually it is an uncommon tree with the exception of the forest – savannah boundary in Uganda where it may be dominant. *Maesopsis eminii* is a large African tropical forest tree introduced to various parts of the tropics for timber production or as a shade tree (common name: umbrella tree) (Sreenivasan and Dharmaraj, 1991). It is naturally regenerating in many places and invasive in the rain forests of the East Usambaras. It is a large canopy tree reaching a height of about 15 m in Western Africa to over 40 m in East Africa, and a diameter up to 1.2 m. The trunk is not buttressed, but is cylindrical and straight, free from branches for 10 to 20 m (see Figure 2.1). It is exceptionally able to live up to 200 years (Normand, 1935). The size and the dentation of the entire leaf exhibit much variation. The species is either deciduous or semi-deciduous depending on local climatic conditions. The sex expression and pollination system is poorly understood but flowers are thought to be hermaphrodite and protogynous and insects are the likely pollination agent. Flowering and fruiting starts after four to ten years and large seed crops are produced every year often every six months. The fruits take six months to mature and change color from green to yellow, red and finally to black when ripe (see Figure 2.2) (Binngeli, 1989). Many insects and fungal diseases affect *M. eminii* causing defoliation, stem breakages and bark cankers.

Maesopsis eminii is used in African traditional medicine, thanks to the diuretic, purgative, emetic and antidiarrhoetic activity of its bark. On the Liberian-Ivory Coast border, this plant is considered violently purgative and to be a secondary diuretic and emetic (causes vomiting) (Kerharo and Bouquet, 1950). The root-bark is used in Cameroon as diuretic for ascites which is an abnormal accumulation of fluid in the abdominal cavity, characterized by painless enlargement of the abdomen (Santesson, 1926). Also in Cameroon, this plant is used in the Sangmelima region of the Southern province as abortifacient (Noumi et al., 2001). The bark-pulp in water is injected into the rectum, or the bark in palm-wine, or the leaf in decoction is drunk. These are antidotes, and very strong purgatives for the obstinate constipation which characterizes the so called "Goat's disease" (Kerharo and Bouquet, 1950). The drastic purgative and emetic effects of the bark of *Maesopsis eminii* are reduced by adding a decoction of fresh palm seeds, or rice water (Kerharo and Bouquet, 1950). In Nigeria, the bark, pounded with salt from the salt-bush tree, dissolved in water is drunk for constipation and for painful menstruation (Ainslie, 1937). Cooper has also mentioned that a bark infusion is used in Liberia to treat "all chronic and latent disease". A leaf decoction is sometimes described as a diuretic and purgative for "yellow fever" (Irvine, 1961).

Figure 2.1: *Maesopsis eminii* forest in Tanzania

Figure 2.2: Fruits and foliage of *Maesopsis eminni*

A previous phytochemical study of the non- polar extract of the bark of this plant reveals only the presence of anthraquinones and some phenolic compounds like musizin (**5**), chrysophanol (**6**), physion (**7**), xanthorin (**8**), islandicin (**9**), cynodontin (**10**), maesopsin (**11**), (Janes et al., 1961; Cumming and Thomson, 1970; Ekpa et al., 1985). According to the fact that most of the plants from the family Rhamnaceae are known as rich sources of not only anthraquinones and phenolics, but also steroids and triterpenoids (Ikan, 1991a), this species was selected to be investigated with respect to its further constituents, as part of a contribution to the phytochemical study of Cameronian medicinal plants.



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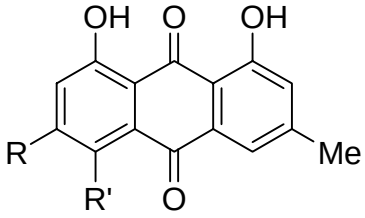

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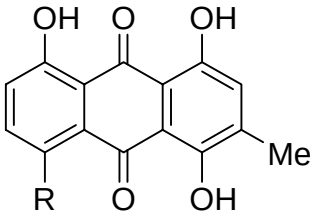

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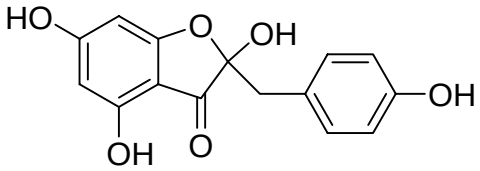

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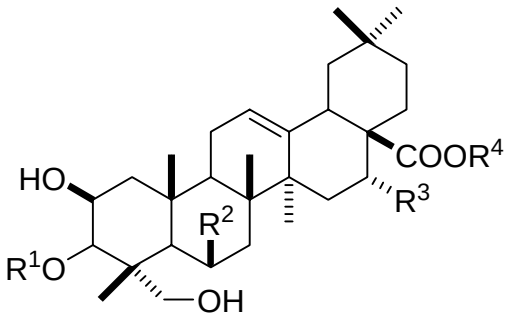
2.1.2 Generalities on the Genus *Autranella* (*Mimusops*)

Autranella is also known as *Mimusops*. The genus *Mimusops* belongs to the family Sapotaceae and comprises 30 species (Jahan et al., 1995), one of which, *Mimusops congolensis* (syn. *Autranella congolensis*), is indigenous to Cameroon. Its other common names are Elanzok, Elang (Cameroon), Kabulungu (Zaire). *M. congolensis* is widely distributed throughout the dense equatorial forests (Cameroon, Congo, Gabon, Nigeria, etc.) (N'Sosso, 1995). The trees are large, attaining heights of 40 to 50 m, with trunk

diameters often about 120 cm, sometimes up to 3 m. This trunk develops straight and cylindrical boles, that are clear of branches for about 30 m. The leaves are glabrous beneath, slightly clustered at the end of the branches, obovate-oblong and shortly acuminate. They are 10 to 15 cm long and 3 to 4.5 cm broad, with numerous fine lateral nerves (Hutchinson and Dalziel, 1963a). The reproductive type is pollination and fertile flowers are hermaphrodite; unisexual flowers are absent. Their fruits are oval with a pointed tip ripening yellow or orange (Pooley, 1993). The heartwood is red to reddish brown with darker streaks; the sapwood, grayish, is not always sharply demarcated from the heartwood. The heartwood is rated as very durable though there may be slight termite attack, resistant to dilute acids, good weathering characteristics, highly impermeable (Chudnoff, 1984). The wood-dust is very irritating to mucous membranes (Anon, 1954), and to the respiratory tract (Orsler, 1973).

Autranella congolensis is used for heavy construction, flooring, furniture and cabinetmaking, acid vats, turnery, joinery (Bolza and Keating, 1972); its seeds are used and traded as rattlers for dancers (African Regional Workshop, 1996). *Mimusops* species are also reported to have considerable reputation in Indian traditional medicine due to their anthelmintic, tonic, and astringent activities (Sahu et al., 1995). The bark and the fruit of *M. elengi* are used in the treatment of diarrhea and chronic dysentery, and a decoction of the bark is used as gargle. The pounded seeds pasted with oil are used for the treatment of obstinate constipation. Pillow stuffing made from the dried flowers induces nasal discharge and relieves headache (Misra et al., 1974).

Previously, several triterpenoid and their saponins, protobassic acid (**12**), Mi-glycoside 1 (**13**), Mimusopside A (**14**), Mimusopside B (**15**), Mi-saponin A (**16**), Arganin C (**17**), Mimusin (**18**), Mimusopgenon (**19**), Mimugenon (**20**), β-amyrin (**21**), Taraxerol (**22**), Lupeol (**23**) (Sen et al., 1995; Sahu, 1996, Sahu et al., 1995, 1997; Lavaud et al., 1996; Srivastava and Singh, 1994; Eskander et al., 2005), steroids and stroidal glycosides, β-sitosterol (**24**), sitosteryl β-D-glucopyranoside (**25**), (*24R*)-stigmast-7,22-(*E*)-dien-3α-ol (**26**), (*24R*)-stigmast-7,22-(*E*)-dien-3α-ol β-D-glucopyranoside (**27**), (*24R*)-stigmast-7,22-(*E*)-dien-3α-ol β-D-galactopyranoside (**28**) (Misra et al, 1970; Jahan et al., 1995) have been found as constituents of this genus. Sahu (1996) also mentioned the presence of taxifolin (**29**) which is a flavonol, in the seeds of *mimusops elengi*. The saponins of fruits were reported to possess anti-inflammatory activity (Bhargava et al., 1970), and those fom the bark of *M. elengi* exhibited antiulcer activity (Shah et al., 2003).



12 $R^1 = R^2 = R^3 = H$

13 R^1 = Glc; R^2 = OH; R^3 = H

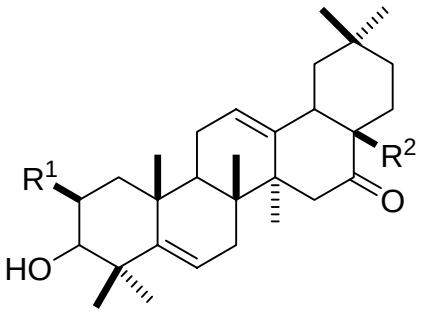
14 R^1 = Glc; R^2 = H; R^3 = H; R^4 = Ara(1→2)Rhm-

15 R^1 = Glc; R^2 = H; R^3 = OH; R^4 = Ara(1→2)Rhm-

16 R^1 = Glc; R^2 = H; R^3 = H; R^4 = Rhm(1→3)Xyl(1→4)Rhm(1→3)Ara-

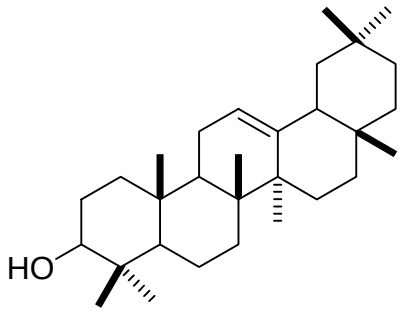
17 R^1 = Glc; R^2 = H; R^3 = OH; R^4 = Rhm(1→3)Xyl(1→4)Rhm(1→3)Ara-

18 R^1 = Glc(1→6)Glc; R^2 = H; R^3 = H; R^4 = Rhm(1→3)Xyl(1→4)Rhm(1→3)Ara-



19 R^1 = OH R^2 = H

20 R^1 = H R^2 = CH_3



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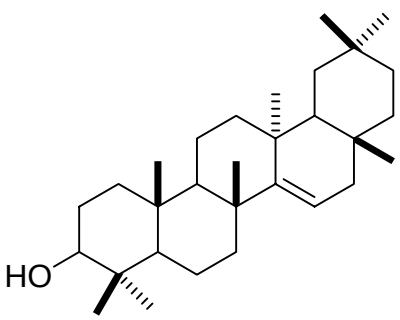

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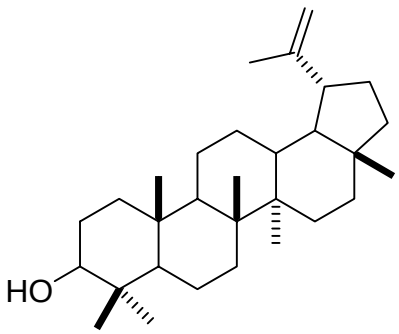

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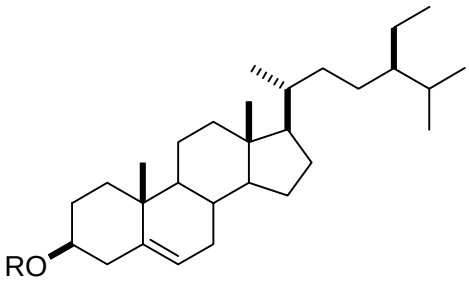
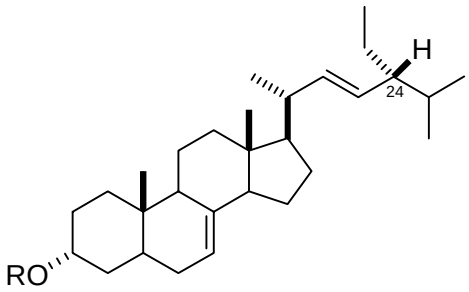


24 R = H

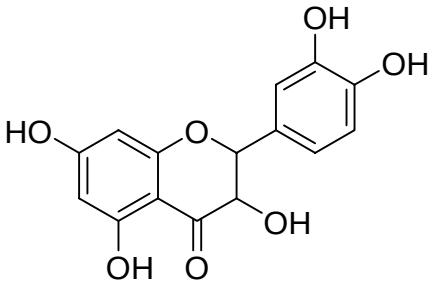
25 R = β-D-Glc



26 R = H

27 R = β-D-Glc

28 R = β-D-Gal



29

Since the chemical constituents of *Mimusops congolensis* (*Autranella congolensis*) have not been previously investigated, the species was selected as part of our research project on plants growing in Cameroon and used in folk medicine.

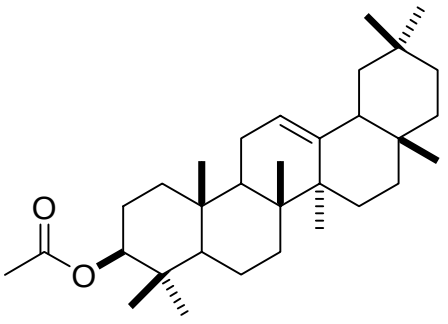
2.1.3 Generalities on the Genus *Pentadesma*

The genus *Pentadesma* belongs to the family Guttiferae. It occurs in closed forest region, especially in evergreen forest. It is common to swampy areas by stream sides. 15 species are known for this genus and are reported in Table 2.1. The species *P. butyracea* and *P. grandifolia* are widely distributed from Guinea to Cameroon. The genus *Pentadesma* consists of large buttressed evergreen trees up to 30 m and more. Sultanbawa mentioned that the species *Pentadesma* Sabine is only confined to Africa (Sultanbawa, 1980). Trees of this genus are easily reared from seed and mature quickly. Their leaves are opposite, grouped at the top of the branches, petiolate, 10 to 22 cm long and 3.5 to 7 cm broad, with mumerous close parallel lateral nerves (Bamps, 1970). Their fruits are broadly ellipsoid, pointed, about 15 cm long and 10 cm broad; they are readily collected. The seeds are large with flattened sides (Hutchinson and Dalziel, 1963b). As they regenerate freely from the

stump and from self-sown seed, they are suitable trees for reafforestation purposes (Irvine, 1961).

Fat is extracted from their seeds, and is used for cooking, soap-making, and on for cosmetics. The timber of *P. butyracea* is good, useful for pit-props and housebuilding, and thought to be fairly resistant to termites. Masts, oars, and canoes can be made from it. The root decoction of *P. butyracea* is a vermifuge in Liberia (Harley, 1941), and the bark is a fish poison in Ghana. The bark infusion is used as lotions for parasitic skin diseases, and when boiled it used for diarrhoea. The fat from the seeds is an unguent for skin and hair (Irvine, 1961).

Among the 15 species enumerated, only *P. butyracea* (Adomako, 1977; Gunasekera et al., 1977a; Rosenthaler, 1928) and *P. kerstingii* (Wagner et al., 1914) have previously been investigated with respect to their secondary metabolites. Triterpenoids, sterol and xanthones were described. From the bark and timber extracts of *P. butyracea* the following compounds have been isolated: β-amyrin acetate (**30**), β-amyrin (**31**), β-sitosterol (**24**), 1,3,5-trihydroxy-2-methoxyxanthone (**32**), jacareubin (**33**), osajaxanthone (**34**) and pentadesmaxanthone (**35**) (Gunasekera et al., 1977a). Xanthones, characteristic secondary metabolites of Guttiferae (Monache et al., 1983), were found to be the major constituents. They have demonstrated various biological activities, such as antibacterial (Iinuma et al., 1996), antiinflammatory (Gopalakrishnan et al., 1980), and antifungal (Gopalakrishnan et al., 1997). On the other hand, Jones described xanthone derivatives to be effective as an allergy inhibitor, bronchodilator in the treatment of asthma (Jones et al., 1977). In this project, in continuation of the phytochemical work on Cameroonian medicinal plants, the constituents of the roots of *Pentadesma grandifolia* were examined, of which no chemical investigation for its constituents had been reported previouly.



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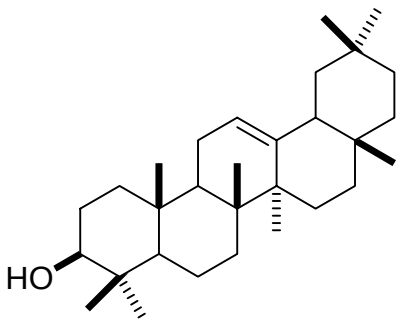

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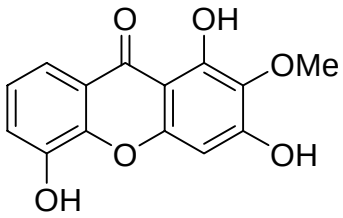

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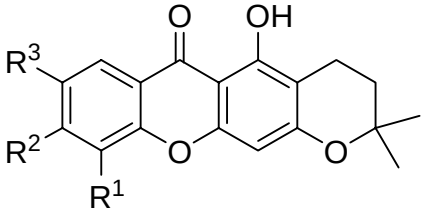

33 $R^1 = R^2 = OH$ $R^3 = H$

34 $R^1 = R^2 = H$ $R^3 = OH$

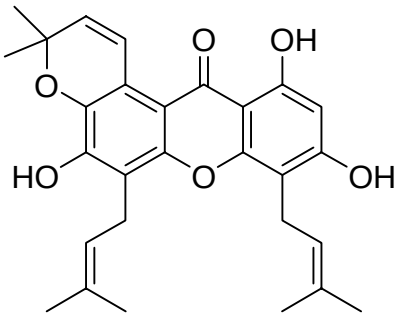

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Table 2.1: Different species of *Pentadesma* identified in Cameroon and in Africa.

No	Species	Authors
1	*Pentadesma grandifolia*	E. G. Baker
2	*Pentadesma butyracea*	Sabine
3	*Pentadesma devredii*	Spirl.
4	*Pentadesma exelliana*	Staner
5	*Pentadesma kerstingii*	Engl.
6	*Pentadesma lebrunii*	Staner
7	*Pentadesma lecomteana*	Pierre ex A. Chevalier
8	*Pentadesma leptonema*	Pierre
9	*Pentadesma nigritana*	E. G. Baker
10	*Pentadesma leucantha*	A. Chevalier
11	*Pentadesma maritima*	Pierre
12	*Pentadesma ogoouensis*	Baud.
13	*Pentadesma parviflora*	Exell.
14	*Pentadesma reyndersii*	Spirl.
15	*Pentadesma rutshuruensis*	Spirl.

2.1.4 Biological activities of the compounds isolated from the three genera

Some compounds isolated from these genera have exhibit some interesting biological activities. For example, lupeol (**23**) exhibited hypotensive activity (Harbone and Baxter, 1993), in vitro antimalarial activity (Alves et al., 1997), as well as anti-inflammatory activity (Geetha and Varalakshmi, 1988). Sitosterol (**24**) and stigmasterol were found to be essential starting materials in pharmaceutical industry where they are used in the manufacture of steroidic drugs (contraceptives and anti-inflammatory agents).

Since the compounds isolated from the three genera belong to several groups of natural products presenting various biological properties, this could explain the wide use of these plants in traditional medicine.

Among the compounds isolated from these genera, most of them belong to the class of pentacyclic triterpenoids. Although medicinal uses of this class of compounds are rather limited, considerable recent work in this regard strongly indicates their great potential as drugs. Moreover, despite the remarkable diversity that is already known to exist among the carbon skeletons of triterpenes, new variants continue to emerge (Mahato et al., 1992).

Taking into account the complexity of the structures of triterpenoids, it seemed necessary to make a review of the literature on this class of natural products, in order to better be able to describe them during this work.

2.2 Properties of pentacyclic triterpenoids and their saponins

Triterpenoids are ubiquitous non steroidal secondary metabolites of terrestrial and marine flora and fauna, occurring in the free form as well as in the forms of ether, ester and glycoside. As the name implies, triterpenoids are isopentenoids composed of thirty carbon atoms. They are built up of six isoprene units and derive from squalene, presumably via ring opening of squalene-2,3-epoxide (**36**) (oxidosqualene), followed by a concerted cyclisation (Figure 2.3) (Abe et al., 1993; Wendt et al., 2000). Because of this biosynthetic origin, they are all hydroxylated at C-3 position. The cyclization proceeds to give a protosteryl or a dammaryl cation which then undergoes a series of 1,2-methyl and hydride shifts with proton elimination. The final product of the cyclization is a pentacyclic triterpene only if the oxidosqualene previously has the so-called “chair-chair-chair”

conformation (Goodwin, 1980; Abe et al., 1993). They may possess acyclic, mono-, di-, tri-, tetra- or pentacyclic carbon skeletons. Pentacyclic triterpenoids are dominant constituents of this class and have been widely investigated (Mahato and Kundu, 1994). We will limit our study to pentacyclic triterpenoids, subdivided in several structural groups.

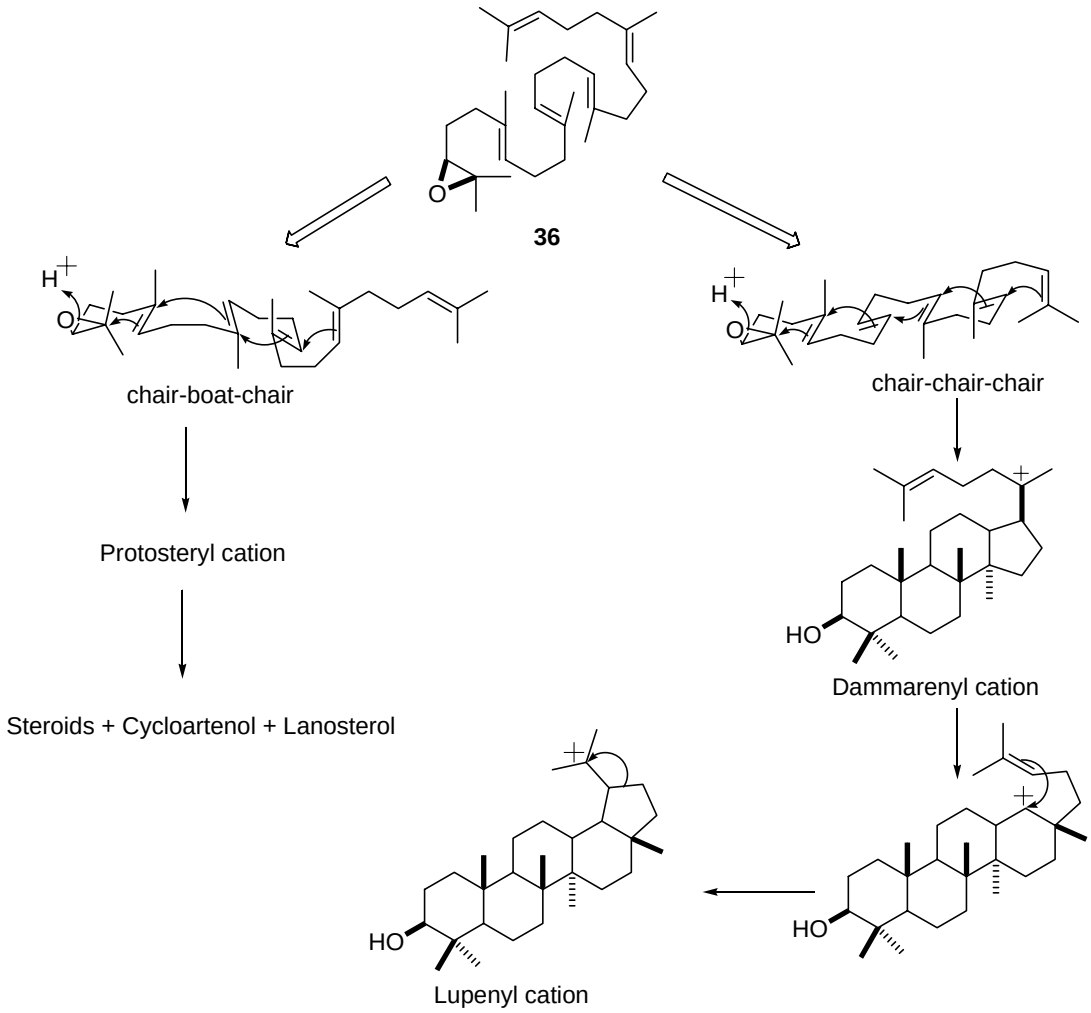


Figure 2.3: Cyclization of oxidosqualene.

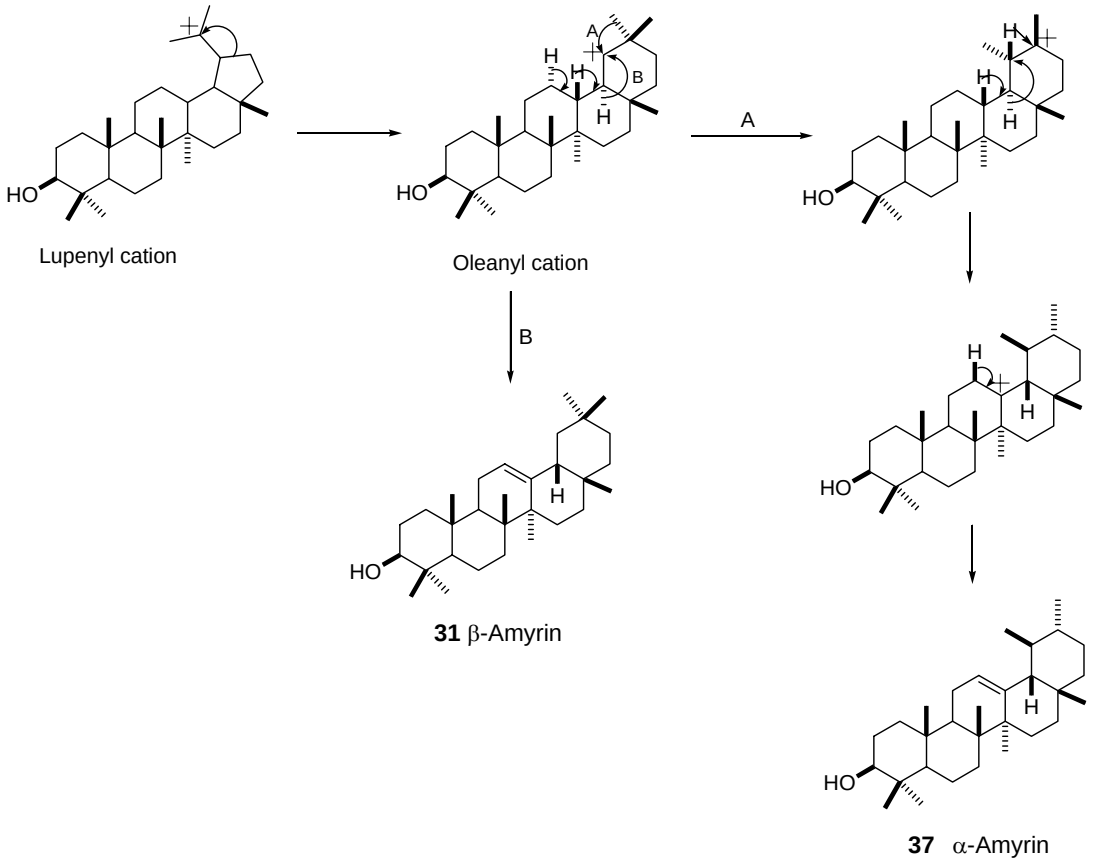
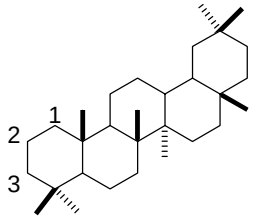


Figure 2.3. continued.

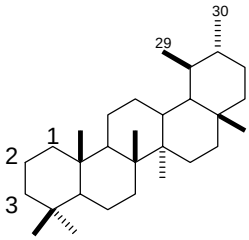
2.2.1 Different structural groups

According to Mahato and coworkers, pentacyclic triterpenoids can be classified into 22 structural groups (Mahato et al., 1994), which are represented in Table 2.2.

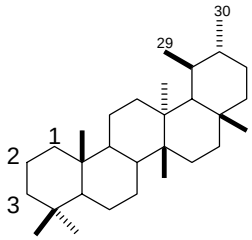
Table 2.2. Different structural groups of pentacyclic triterpenoids.



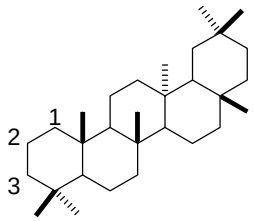
38 Oleanane



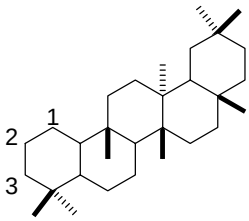
39 Ursane



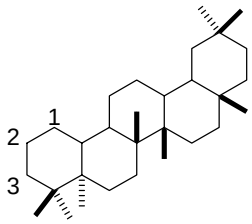
40 Bauerane



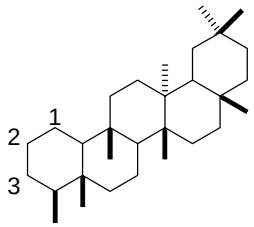
41 Taraxarane



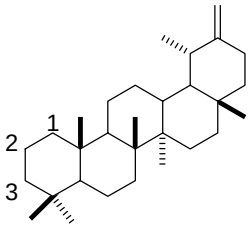
42 Glutinane



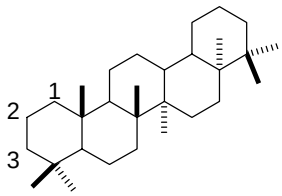
43 Mimusopane



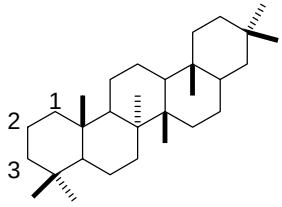
44 Fridelane



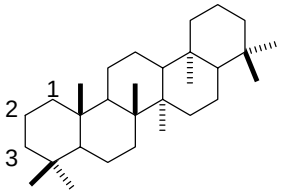
45 Taraxastane



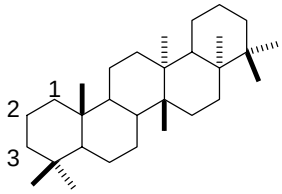
46 Kairatane



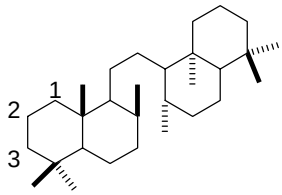
47 Stictane

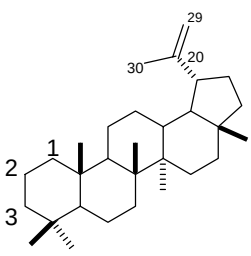


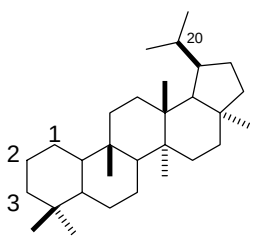
48 Gammacerane



49 Swertane



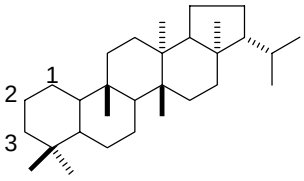




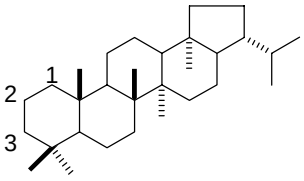
50 Onocerane

51 Lupane

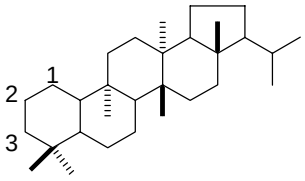
52 Hancokinane



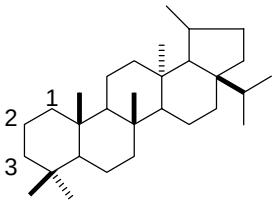
53 Fernane



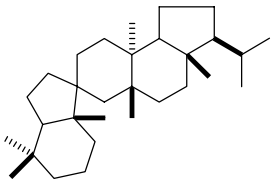
54 Hopane



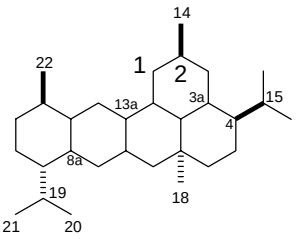
55 Sorghumane



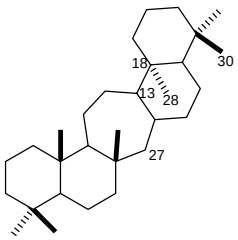
56 Friedomadeirane



57 Spirosupinane



58 Bicadinane



59 Serratane

Triterpenes are normally hydroxylated at C-3 and certain methyl groups are frequently oxidised to hydroxymethyl, aldehyde or carboxyl functionalities. When a sugar moiety is linked to a triterpene, the term saponin is used for the corresponding compound. The aglycone or non-saccharide portion of the saponin molecule is called genin or sapogenin (Hostettmann and Marston, 1995a). The sugar moiety of these saponins are generally oligosaccharide, linear or branched, attached to a hydroxyl or a carboxyl group or both. The site of attachment may be one (monodesmoside), two (bidesmoside) or three (tridesmoside) (Mahato and Kundu, 1994).

2.2.2 Occurrence and distribution

Triterpenoids and their saponins are extremely widely distributed in the plant kingdom. Some plants contain large quantities of triterpenes in their latex and resins, and among their physiological functions a chemical defence against pathogens and herbivores has been proposed (Brown, 1998). These plant's constituents content depends on factors such as the cultivar, the age, the physiological state and the geographical location of the plant. There can be considerable variation in composition and quantity of triterpenoids and saponins in vegetable material from different places (Boiteau et al., 1964a, Hostettmann and Marston, 1995b).

2.2.3 Isolation procedure

The isolation methods for triterpenoids are based on the extraction of the plant material with duly selected organic solvents. Generally, to this end petrol ether, ethyl ether and ethanol are employed. More rarely, benzene, carbon tetrachloride, acetone, methanol or chloroform are used (Boiteau et al., 1964b). Obtention of pure componenents is accomplished by chromatographic techniques. The current general procedures for obtaining crude saponin mixture were reported as follows:

- extraction with methanol, ethanol, water or aqueous alcohol;
- a defatting step (generally with petroleum ether); this can be performed before the extraction step or on the extract itself;
- extracts are dissolved or suspended in water and shaken with n-butanol saturated with water.

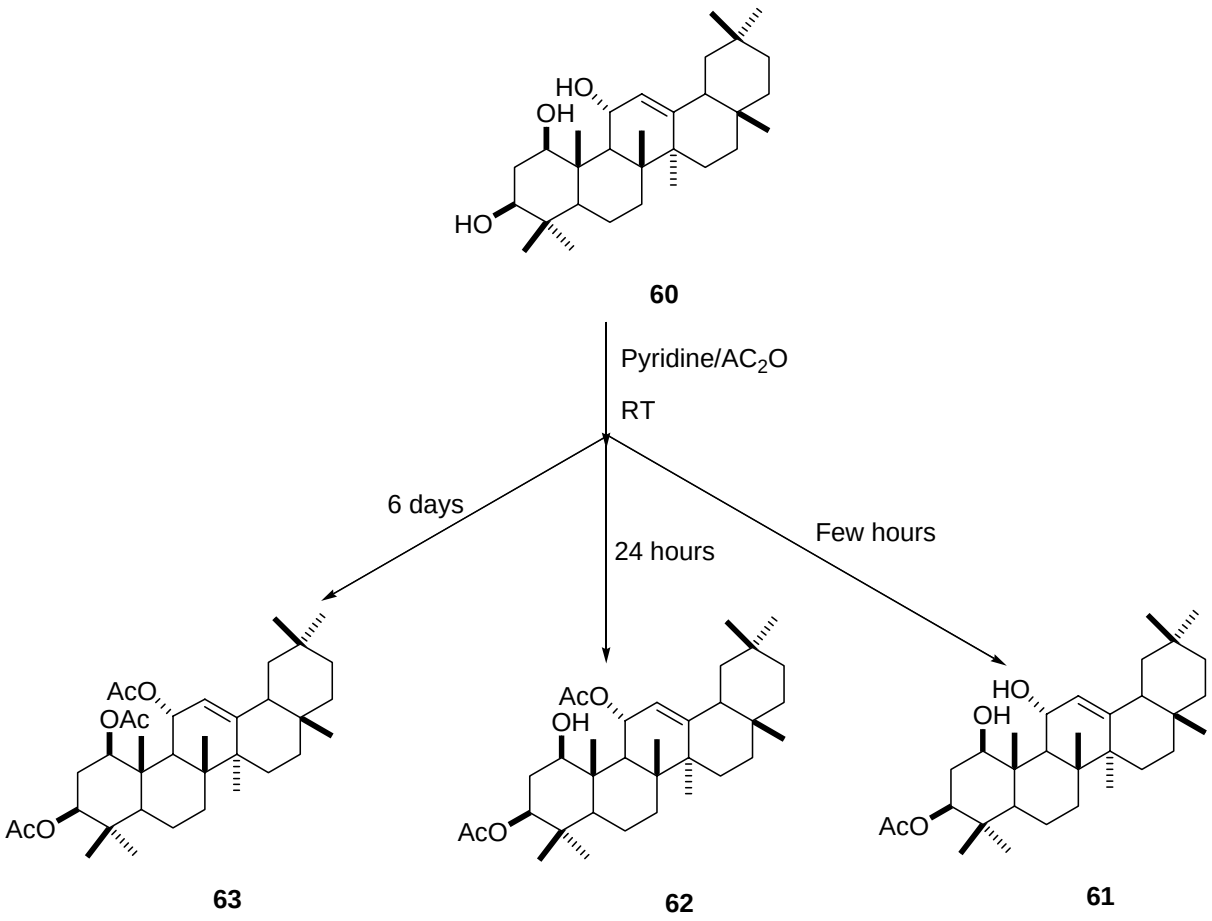
The isolation of pure saponins requires one or (as almost always the case) more chromatographic separation Steps (Hostettmann and Marston, 1995c).

2.2.4 Typical chemical reactions on pentacylic triterpenes

2.2.4.1 Acetylation

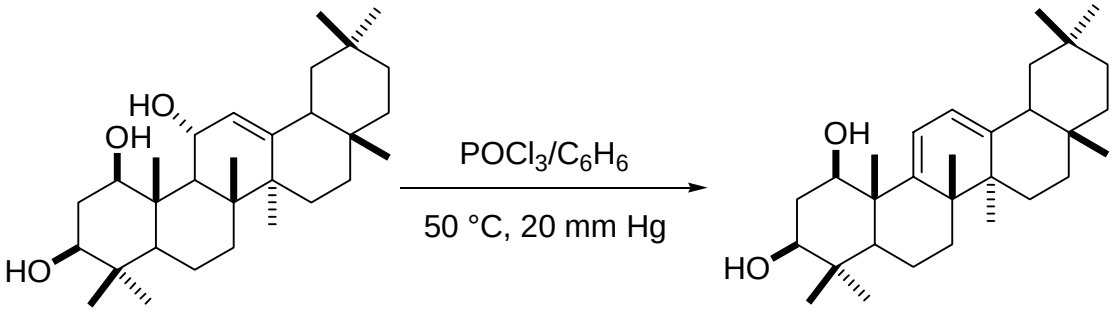
This reaction is performed in a sealed tube at room temperature in a mixture of pyridine and acetic anhydride. It takes a short time for free hydroxyl groups and a long time for sterically hindred hydroxyl groups. An example has been decribed by Gonzalez and coworkers, where compound (**60**) was treated with a mixture of pyridine and acetic anhydride at room temperature for a few hours to yield (**61**). When the solution was kept at

room temperature for 24 hours, compounds (**61**) and (**62**) were obtained. When the same process was followed for 6 days, (**61**), (**62**) and (**63**) were obtained (Gonzalez et al., 1987).



2.2.4.2 Dehydration

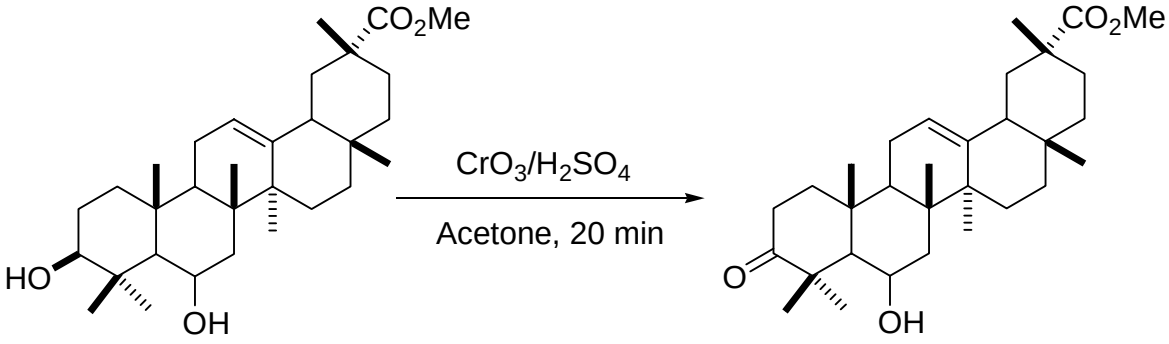
This is a reaction by which triterpenic alcohols can be converted to their corresponding alkenes. It can be performed in different manners. Gonzalez and coworkers dissolved compound (**60**) in dry benzene. When evaporated to dryness at 50 °C and 20 mm pressure, compound (**64**) was obtained after purification of the mixture (Gonzalez et al., 1987).



60 **64**

2.2.4.3 Oxidation of the hydroxyl group

This is also a characteristic reaction in triterpenoids, accomplished by Jones's reagent (CrO_3 in aq.H_2SO_4). The example below was reported by Ngounou and coworkers (Ngounou et al., 1988).



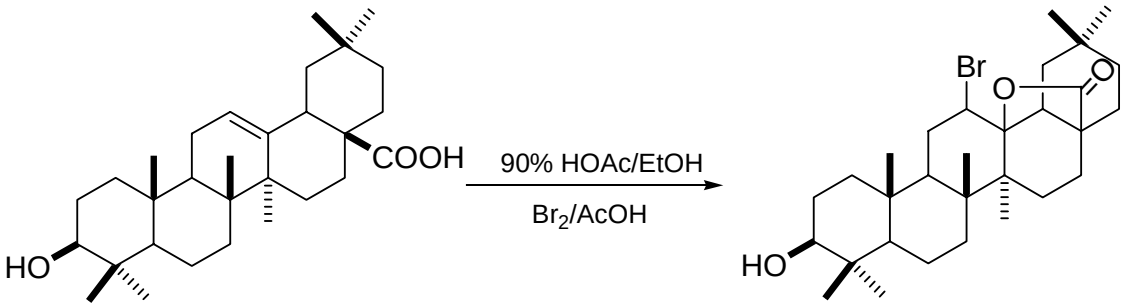
65 **66**

2.2.4.4 Methylation of the carboxy group

Methylation of a carboxy group may be performed using diazomethane in ether at room temperature. This reaction is reported for the methylation of a mixture of acids in order to facilitate their separation by chromatography (Caputo et al., 1974).

2.2.4.5 Bromination or bromolactonization of the double bond

This method involves the treatment of the product with bromine in acetic acid. It was reported as a method for the separation of substituted olean-12-en-28-oic acids from the corresponding urs-12-en-28-oic acid isomers (Lewis and Tucker, 1983). Members of the ursene family were reported to be inert under the conditions used. Thus a mixture of ursolic acid and oleanolic acid was dissolved in 90% HOAc-EtOH and treated with bromine in acetic acid to give a mixture of bromolactone of oleanolic acid and unreacted ursolic acid. This mixture was separated by solvent extraction or chromatography (Lewis and Tucker, 1983). Example:



67 **68**

2.2.4.6 Reduction reactions giving alcohol groups

The most commonly used reagents are $NaBH_4$ and $LiAlH_4$. $LiAlH_4$ transforms ketone, aldehyde, acid and ester triterpenoids to their corresponding hydroxy-triterpenoids. $NaBH_4$ is more selective since it transforms only aldehyde and ketone to an alcohol.

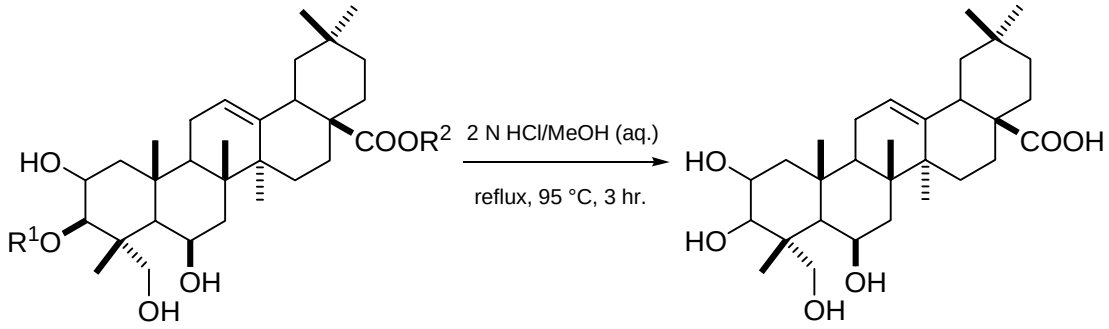
2.2.4.7 Basic hydrolysis

Cleavage of *O*-acyl glycosidic sugar chains is achieved under basic hydrolysis conditions, typically by refluxing with 0.5 M potassium hydroxide (Domon and Hostettmann, 1984; Kotchetkov and Khorlin, 1966). Alternatively, 1-20% ethanolic or methanolic solutions of potassium hydroxide may be used but there is a risk of methylation, especially of the carboxyl groups of triterpene acids (Hostettmann and Marston, 1995d).

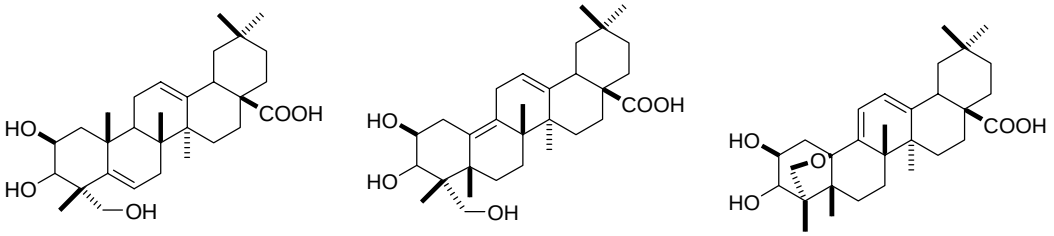
2.2.4.8 Acidic hydrolysis

Acidic hydrolysis is carried out by refluxing the saponin in acid for a certain time, typically with 2-4 M hydrochloric acid for 4 hours. The aqueous solution remaining is extracted with diethyl ether, chloroform or ethyl acetate to obtain the aglycone. Extraction of the sugars from the aqueous layer is performed with pyridine, after neutralizing the solution and evaporation to dryness. The saponins are completely cleaved into their constituents by this method. Hence, information is obtained on the identity of the aglycone and the number and nature of monosaccharides present (Hostettmann and Marston, 1995e). If a prosapogenin (obtained after cleavage of an ester linkage by basic hydrolysis) is acid hydrolysed, the nature of the sugar chains which are ether-linked to the aglycone can be established. An aqueous reaction medium can be replaced by alcohol or dioxane (Hostettmann and Marston, 1995e).

An example is the acid hydrolysis of mimusopside A (**14**, R^1 = Glc, R^2 = Ara-Rhm-), which yielded the genuine aglycone, protabassic acid (**12**) and three acid-catalysed rearranged aglycones, bassic acid (**12a**), mimusopic acid (**12b**) and mimusopsic acid (**12c**) (Sahu, 1996).



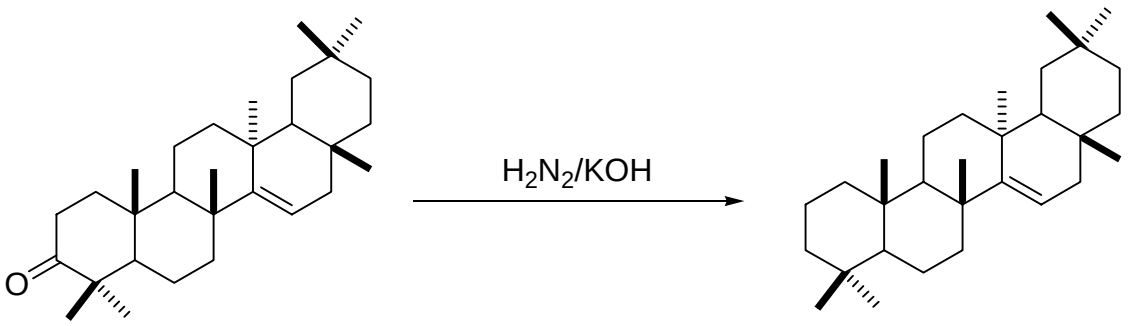
14 **12**



12a **12b** **12c**

2.2.4.9 Reduction reactions giving a methylene group

The reduction of aldehydes and ketones to alkanes is an important reaction used for the determination of the position of carbonyl groups in triterpenic ketones and aldehydes. Three reagents are usually used for this reduction, Wolff-Kishner reagent, Huang-Minlon reagent and Clemmensen reagent (Boiteau et al.,1964c). With the Wolff-Kishner reagent and Huang-Minlon reagent, the ketone function of base-stable compounds, whatever its position, is reduced and replaced by a CH_2. This method is generally preferable for triterpenoids to the Clemmensen reduction, method which is not applicable to acid-sensitive substrates and often causes isomerizations by displacement of the double bonds (Boiteau et al.,1964c).



69 **70**

2.2.5 Structure determination

Structure determination of triterpenoids and their saponins requires a combination of methods in order to arrive at a final conclusion for the structure. Recourse to NMR spectroscopy and mass spectrometry are essential in the investigations of these compounds. In the case of complex saponins, innovations in these techniques are needed for further advances in the investigations (Hostettmann and Marston, 1995f).

2.2.5.1 Mass spectrometry

Mass spectrometry has been used as an important tool for structure determination of triterpenoids for three decades (Shiojima et al., 1992). The choice of ionization methods in MS depends on the polarity, lability, and molecular weight of the compound to be

analysed. The so-called “soft“ ionization techniques such as FAB, ESI and desorption/chemical ionization (D/CI) are employed to obtain molecular weight and sugar sequence information for naturally occuring glycosides (Wolfender et al., 1992; Hostettmann and Marston, 1995f).

The electronic impact MS (EI-MS) is particularly applied to the structure elucidation of triterpenoids and the aglycones obtained from saponins. It is possible to observe the molecular peak and also to arrive at conclusions about the structure of the terpenoid skeleton from the fragmentation pattern. One of the diagnostically important features of the EI-MS of terpenoids is the retro-Diels-Alder (RDA) reaction which cleaves the molecule at the ring containing a double bond and enables information to be furnished about the substitution in the ring systems (Djerassi et al., 1962; Budzikiewicz et al., 1963; Hostettmann and Marston, 1995f). Examples of RDA reaction are in part 4 of this thesis (see Figure 4.3 and Figure 4.7).

The high resolution mass spectrometry (HR-MS) is applied for the establishment of the exact molecular formula since it gives information about the elemental composition through exact mass measurements.

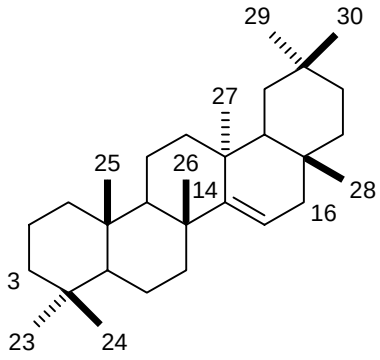
2.2.5.2 NMR spectroscopy

NMR spectroscopy, particularly ^{13}C NMR spectroscopy is now being frequently employed in triterpenoid structure elucidation using various methods of signal assignment, e.g. attached proton test (APT), DEPT, and 2D-NMR spectroscopy (Das and Mahato, 1983; Mahato et al., 1992).

2.2.5.2.1 ^{1}H NMR

The ^{1}H NMR spectra of pentacyclic triterpenoids are highly complex and tedious to analyse. However, the methyl peaks of triterpenes are readily discernible as sharp singlet or doublet absoptions, and most proton resonance positions in oleanene, ursene and related skeletons have been assigned since the 1960s (Kojima and Ogura, 1989) by a variety of techniques. Generally, the resonances of the eight methyl groups in triterpenes are observed in the region δ 0.5 to δ 2.0 (Ageta and Arai, 1983). These chemical shifts were demonstrated to be affected by the introduction of various substituents on the carbon

skeleton (Karliner and Djerassi, 1966; Tursch et al., 1967; Kojima and Ogura, 1989). An example is given in Table 2.3 (adapted from Ageta and Arai, 1983).



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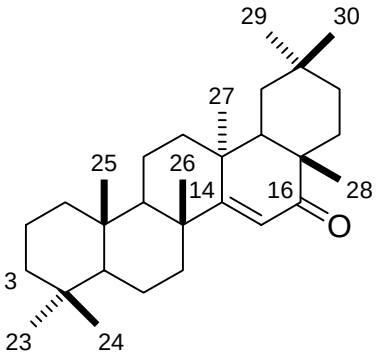

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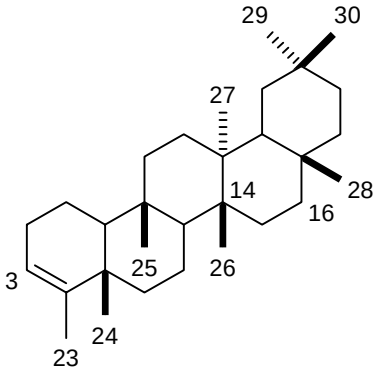

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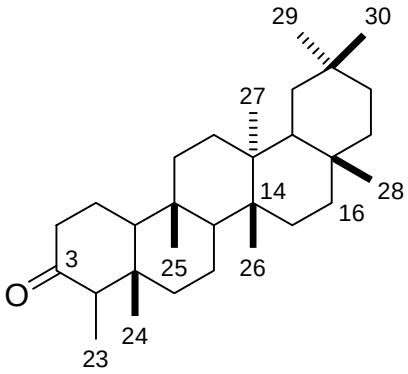

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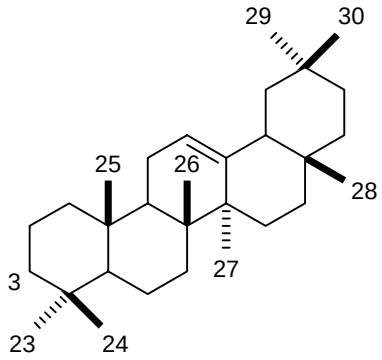

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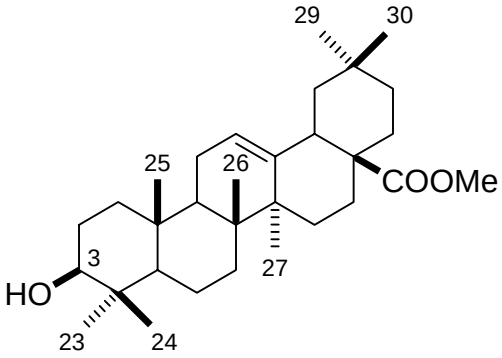


75

Table 2.3: ^{1}H NMR chemical shifts of Methyl groups for coumpounds **70-75** in $CDCl_3$

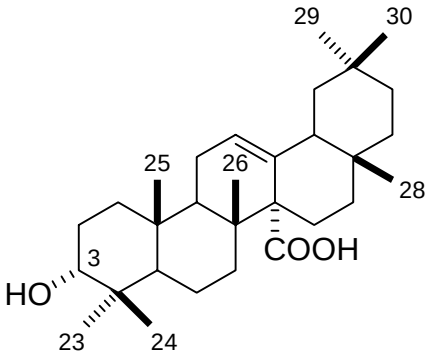
Compound	Methyl signals								Series
	23	24	25	26	27	28	29	30	
70	0.85	0.83	0.91	1.09	0.95	0.83	0.91	0.91	Taraxarane
71	0.87	0.84	0.95	1.14	1.13	1.00	0.97	0.97	
72	1.58	1.00	0.86	1.00	1.00	1.17	0.95	1.00	Friedelane
73	0.88	0.73	0.88	1.01	1.05	1.18	0.97	1.01	
74	0.87	0.82	0.93	0.97	1.14	0.83	0.87	0.87	Oleanane
75	1.00	0.79	0.93	0.74	1.17	-	0.95	0.95	

When difficulties arise in determining the configurations of hydroxyl groups at C-2, C-3 and C-23, C-24 of oleanene and ursene triterpenes, analysis of the ^{1}H NMR signal peaks of the protons on oxygen-bearing carbon atoms gives valuable information (Kojima and Ogura, 1989). For example, the distances ($\Delta\delta_{2\text{-}3}$) between the chemical shifts of H-2 and H-3 are 0.88 ppm in 2β,3β-$(OH)_2$ and 0.7 ppm in 2β,3β-$(OAc)_2$>0.69 ppm in 2α,3β-$(OH)_2$ and 0.35 ppm in 2α,3β-$(OAc)_2$>0.57 ppm in 2α,3α-$(OH)_2$ and 0.27 ppm 2α,3α-$(OAc)_2$>0.12 ppm in 2β,3α-$(OH)_2$ and –0.08 ppm in 2β,3α-$(OAc)_2$. The figures for the dihydroxy compounds are larger (ca 0.2 to 0.3 ppm) than those of the corresponding diacetates because of the lower downfield shift of the H-3 on acetylation (Kojima and Ogura, 1989).

In the case of saponins, the vast majority of proton resonances of the carbohydrate moiety appear in a very small spectral width of 3.0-4.2 ppm, associated with problems of overlapping signals (Hostettmann and Marston, 1995f). However, some useful data can be obtained from ^{1}H NMR spectra for the anomeric configurations and linkages of the sugar chain. For example, the coupling constant of the C-1 proton of α–linked glucose units is approximately 3 Hz, while β–linked units have a coupling constant of 6-7 Hz (Hostettmann and Marston, 1995f). More details on the coupling constant of anomeric sugar protons can be found elsewhere (Kizu and Tomimori, 1982; Agrawal, 1982).

2.2.5.2.2 ^{13}C NMR

Carbon-13 NMR spectroscopy is now widely used for the structure determination of triterpenoids using various methods of signal assigment, but requires relatively large quantities of sample. For assigning chemicals shifts, it is very hepful to compare observed data with data reported for model and related compounds. Compilations of assignments of ^{13}C NMR signal for oleanane (Patra et al, 1981), ursane, lupane, hopane (Wenkert et al., 1978) triterpenes and recently a compilation of the ^{13}C NMR data of selected varieties of naturally occuring pentacyclic triterpenes (Mahato and Kundu, 1994) have been published. The ^{13}C chemical shifts were demonstrated to be affected by the introduction of various substituents on the carbon skeleton like in the ^{1}H NMR spectra. For example, Mahato and Kundu (1994) found that introduction of a hydroxyl group results in downfield shifts of 34-50 ppm for α–carbons and 2-10 ppm for β–carbons and upfield shifts of 0-9 ppm for γ–carbons (Mahato and Kundu, 1994). The substituent effect on chemical shifts of the carbinyl carbon atom depends also to the stereochemistry, and the number of γ–gauche carbons bearing hydrogen atoms able to interact with the hydroxyl group, as well as the number of 1,3-diaxial interactions of the hydroxyl group with carbon atoms. When 1,3-diaxial interactions are absent, the carbinyl carbon is less shielded in the equatorial epimer than in the axial one, because of 1,3-diaxial interactions (Mahato and Kundu, 1994). For example, a comparison of the ^{13}C data of triterpenes (**31**) and (**76**) (see Table 2.4), containing equatorial and axial hydroxyl groups respectively at C-3, reveals that not only the carbinyl carbon of the equatorial isomer is less shielded (δ 79.0) than the axial one (δ 76.4), but also the axial C-4 methyl and C-1 methylene groups in (**31**) are shifted by about 6.5 ppm and 2.0 ppm, respectively, in comparison to that of triterpene (**76**) due to γ–gauche interaction (Mahato and Kundu, 1994).



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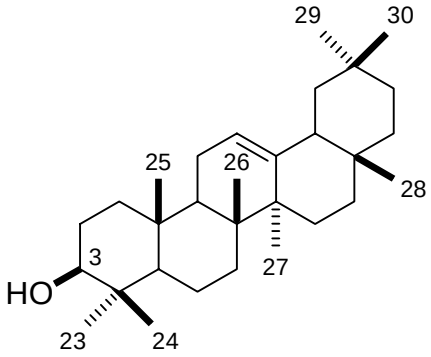

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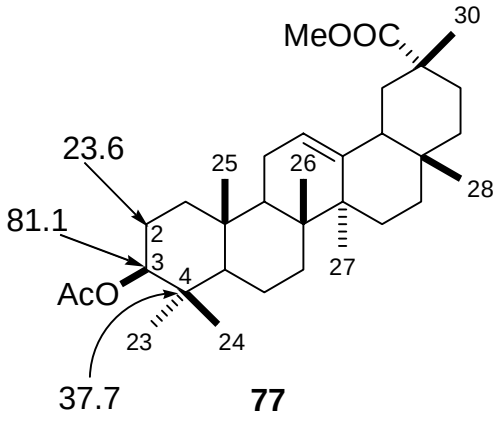

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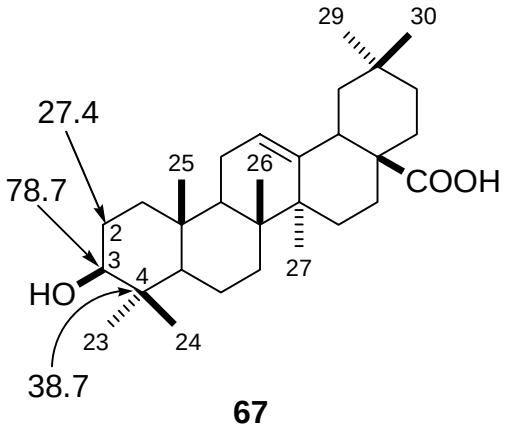


67

The location of the primary hydroxyl group at C-23, C-24, C-29 and C-30 in oleanenes may be determined from the chemical shifts of the hydroxymethylene carbons, as the equatorial hydroxymethylenes (C-23 and C-29) are less shielded than their axial counterparts (C-24 and C-30) (Mahato and Kundu, 1994).

Acetylation of the hydroxyl group accentuates the α–effect and diminishes the β–effect, the latter being attributed to the γ–effect of the acetyl moiety; the γ–effects, however, remain more or less unaltered (see example, in triterpene (**67**) and (**77**)). That means that C-3 is deshielded, while C-2 and C-4, respectively, are shielded. More details on the substituent effects can be found in the review on ^{13}C NMR spectra of pentacyclic triterpenoids (Mahato and Kundu, 1994).

Finally, NMR spectroscopy effectively provides the most complete information and analysis of the spectra allows conclusion to be drawn about - the presence of substituents (hydroxy, carboxyl,…groups), - the number of these substituents, - the number of methyl groups, - the presence of double bond from which the terpenoid's serie can be deduced (by

comparison of the observed chemical shifts of the carbons of the double bond with data reported for model and related compounds).

Table 2.4: ^{13}C NMR data of compounds (**31**), (**67**), (**76**) and (**77**) (adapted from Mahato and Kundu, 1994).

Atom No	**31**	**76**	**67**	**77**
1	38.7	36.7	38.5	38.4
2	27.3	27.6	27.4	23.6
3	79.0	76.4	78.7	81.1
4	38.8	39.9	38.7	37.8
5	55.3	48.8	55.2	55.4
6	18.5	18.0	18.3	18.3
7	32.8	32.9	32.6	32.7
8	38.8	36.7	39.3	39.9
9	47.7	48.8	47.6	47.6
10	37.6	36.7	37.0	37.0
11	23.6	23.6	23.1	23.6
12	121.8	126.0	122.1	123.0
13	145.1	137.6	143.4	144.4
14	41.8	56.0	41.6	41.7
15	26.2	22.7	27.7	26.6
16	27.0	26.2	23.4	27.0
17	32.5	32.9	46.6	32.5
18	47.4	47.0	41.3	46.1
19	46.9	44.2	45.8	40.6
20	31.1	31.0	30.6	42.8
21	34.8	34.2	33.8	29.1
22	37.2	36.2	32.3	36.0
23	28.2	28.2	28.1	28.3
24	15.5	22.1	15.6	16.8
25	15.6	15.8	15.3	15.6
26	16.9	18.0	16.8	16.8
27	26.0	179.0	26.0	26.0
28	28.4	28.2	181.0	28.2
29	33.3	32.9	33.1	179.6
30	23.7	23.6	23.6	19.4

For saponins, analysis of the spectra allows conclusions to be drawn on the six aspects:

(1) positions of attachment of the glycosidic chains to the aglycone,

(2) sequence, nature and number of monosaccharides,

(3) configuration and conformation of the interglycosidic linkages,
(4) presence of acylglycosides in the chains,
(5) nature of the aglycone, and
(6) structures of attached ester acids (Hostettmann and Marston, 1995f).

The glycosylation of a hydroxy group, depending upon its nature (alcoholic and carboxylic), causes a change in chemical shifts at the α- and β-carbons and rarely, γ-carbons relative to the OH group, in which the glycosylation takes place (Mahato and Kundu, 1994). It is of interest that sugar carbon resonces occur largely in a region distinct from that of the sapogenin moiety. The identification of ^{13}C NMR signals belonging to each monosaccharide residue relies mostly on comparison with those of model compounds (Chemli et al., 1987). Chemli and coworkers also proposed a number of simple, general rules for saponin analysis:

(a) the anomeric carbon atoms in pyranoses and in their derivatives resonate at lowest field (90-110 ppm),
(b) carbon atoms bearing secondary hydroxyl groups in pyranoses give signal at 65-85 ppm; signals of alkoxylated carbon atoms are shifted 5-10 ppm to lower field when compared with the corresponding hydroxy-substituted carbon atoms,
(c) carbon atoms carrying primary hydroxy groups are found at 60-64 ppm,
(d) acylation of oxygen leads to smaller (1.5-4 ppm) high frequency shifts of the β-carbon atom (Chemli et al.,1987).

Table 2.5 gives the ^{13}C NMR data for methyl glycopyranosides.

Table 2.5: ^{13}C NMR data for methyl glycopyranosides.

Sugar	C-1	C-2	C-3	C-4	C-5	C-6
β-D-Glc	104.0	74.1	76.8	70.6	76.8	61.8
α-D-Glc	100.0	72.2	74.1	70.6	72.5	61.6
β-D-Gal	104.5	71.7	73.8	69.7	76.0	62.0
α-D-Gal	100.1	69.2	70.5	70.2	71.6	62.2
β-D-Man	102.3	71.7	74.5	68.4	77.6	62.6
α-D-Man	102.2	71.4	72.1	68.3	73.9	62.5
β-L-Rha	102.4	71.8	74.1	73.4	73.4	17.9
α-L-Rha	102.1	71.2	71.5	73.3	69.5	17.9
β-L-Fuc	97.2	72.7	73.9	72.4	71.6	16.3
α-L-Fuc	93.1	69.1	70.3	72.8	67.1	16.3

Methylglycosides were measured in D_2O (Agrawal, 1992).

2.2.5.2.3 Distortionless enhancement by polarisation transfer (DEPT)

This ^{13}C NMR technique is used to enhance the intensities of carbon signals and determine the multiplicities of carbon atom with the aim to differentiate between CH_3, CH_2 and CH signal.

2.2.5.2.4 2D-NMR Spectroscopy

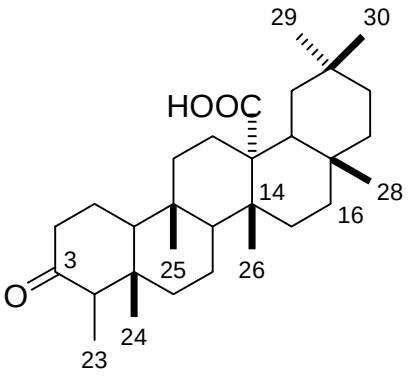
The above mentioned techniques are found to be inadequate for unambiguous assignment of the ^{13}C resonances of compounds possessing complex structural features. A recourse to 2D-NMR techniques is therefore necessary. There are many variants for the techniques of 2D-NMR. Details on these techniques and their applications can be obtained in different NMR spectroscopy books and reviews. Some examples are:

Homonuclear correlation spectroscopy. ^{1}H -^{1}H COSY is one of the most widely used 2D-NMR experiments for ^{1}H assignment. It used to determine vicinal and geminal ^{1}H-^{1}H couplings in molecules.Once the definitive ^{1}H NMR assignments are achieved by

homonuclear COSY or its variant, these can be correlated via ^{1}H-^{13}C COSY spectrum to assign ^{13}C signals (Mahato and Kundu, 1994). As such, ^{1}H-^{1}H COSY not only provides information for unambiguous ^{1}H NMR assignments but also helps in ^{13}C NMR assignments.

Heteronuclear correlation spectroscopy (HETCOR). ^{13}C-^{1}H correlated spectroscopy is one of the most powerful 2D experiments (Mahato and Kundu, 1994). It provides connectivities between ^{13}C and ^{1}H signals and also gives the number of hydrogen atoms attached to each individual ^{13}C atom.

Heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond connectivity (HMBC) are reported as the experiments of choice at present. The HMQC experiment is a proton-detected heteronuclear chemical shift correlations technique. The HMBC experiment is an inverse-detected 2D-NMR technique used to detect connectivities mediated by two- or three-bonds (frequently) and provides important information about the molecular structure from the connectivities. As triterpenes have many methyl groups, the HMBC experiment is very useful for assigning ^{13}C NMR data of these compounds (Mahato and Kundu, 1994). For example, the methyl doublet of trichadonic acid (**78**) was found by means of the proton detected direct C-H coupling (HMQC) study, to be highly shielded, which is typical for the 23-Me resonance of a 3-oxo-friedelane (Mahato and Kundu, 1994). Assuming this resonance assignable to C-23, the application of the HMBC technique led to the elucidation of the structure and ^{13}C assignments of this triterpene (**78**) by identifying ^{2}J and ^{3}J connectivities associated with the methyl proton resonances (Mahato and Kundu, 1994).



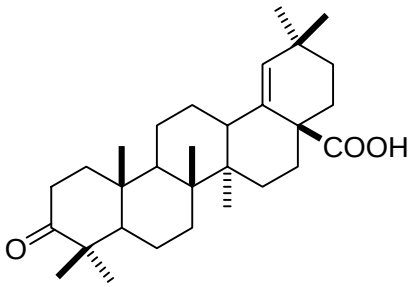
78

2.2.6 Physiological properties of pentacylic triterpenoids and their saponins

The wide occurrence in nature and the structural diversity of triterpenoids have always attracted attention for evaluation of their biological activities. The list of biological activities associated with triterpenoids and their saponins is very long (Mahato et al., 1992; Hostettmann and Marston, 1995g).

2.2.6.1 Antitumour and anticancer activity

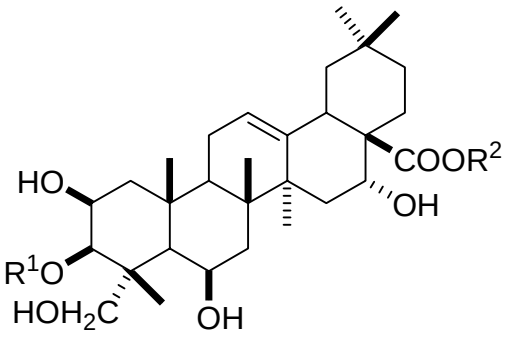
The relation between chemical structure and anticancer, antitumour and anti-HIV activities of some pentacyclic triterpenoids was studied (Mahato et al., 1992; Hostettmann and Marston, 1995e, Ito et al., 2001). Epimanidiol (3β,16α-dihydroxy-olean-12-ene) was found to be cytotoxic at 0.226 mmol/L (100 μg/mL) against the following human cancer cell lines: HEC-1-A, CAMA-1, ME-180, u-87MG, CALAU-1 and SK-OV-3. Several other pentacyclic triterpenoids were tested by Mahato and coworkers and they came out with the conclusion that the presence of a 16α-hydroxy group is important for the appearance of cytotoxicity (Mahato et al., 1992). Oleanolic acid and their derivatives were also reported to be active against the tumour promotor 12-*O*-tetradecanoyl-phorbol 13-acetate (TPA) (Mahato et al., 1992). Several triterpenoids have also been reported to show anti-HIV (Hashimoto et al., 1997; Kashiwada et al., 1998). For example, moronic acid (**79**) showed significant anti-HIV activity, EC_{50}<0.22 μM (<0.1 μg/mL) by suppressing of the proliferation of HIV infected T cells (H9) (Ito et al., 2001).



79

2.2.6.2 Anti-inflammatory activity

Triterpenoids are known to exhibit good anti-inflammatory activity (Safayhi and Salier, 1997). In fact, glycyrrhetinic acid (3β-hydroxy-11-oxo-olean-12-en-30-oic acid) inhibited carragenin-induced edema in the rat paw and inhibited leukocyte migration in the pleural space induced by dextran injection (Mahato et al., 1992). Mahato and coworkers also demonstrated that dihemiphthalate derivatives of 18β-olean-12-ene-3β,30-diol, 18β-olean-9(11),12-diene-3β,30-diol and olean-11,13(18)-diene-3β,30-diol showed a strong inhibition of ear edema (Mahato et al., 1992). The saponins from the roots and bark of *Crossopteryx febrifuga* (Rubiaceae), crossoptine A (**80**) and crossoptine B (**81**) have anti-inflammatory, mucolitic, antioedemic activities (Gariboldi et al., 1990; Hostettmann and Marston, 1995g).



80 R^1 = Glc R^2 = α-L-Rhm(1→3)Xyl(1→4)α-L-Rhm(1→2)Ara-

81 R^1 = Api(1→3)Glc- R^2 = α-L-Rhm(1→3)Xyl(1→4)α-L-Rhm(1→2)Ara-

2.2.6.3 Action on metabolism

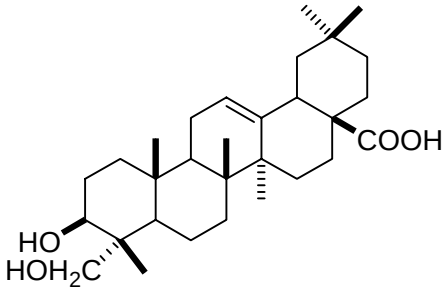
Das and Mahato reported that ursolic acid and its derivatives decreased the blood cholesterol, β-lipoprotein and phospholipid concentrations in rabits (Das and Mahato, 1983). The decrease in activation of cytooprotective PG synthesis whitin the gastric mucosa might contribute to the ulcer healing effect of carbenoxolone (11-oxo-oloean-12-en-30-oic acid 3β-succinate) (Das and Mahato, 1983). Glycyrrhetinic acid has hypolipemic and antiatherosclerotic activity greater than the established antiatherosclerotic polysponin (Mahato et al., 1992).

2.2.6.4 Antimicrobial activity

The antifungal activity of some pentacyclic triterpenoids was tested in vitro using *Saccharomyces carlsbergenesis* as test organism and it was found that the pentacyclic triterpene glycosides of oleanolic acid and hederagenin (**82**), with free carboxylic group at C-28 and C-27, possess the highest fungicidal activity (Das and Mahato, 1983).

2.2.6.5 Effect on biosynthesis

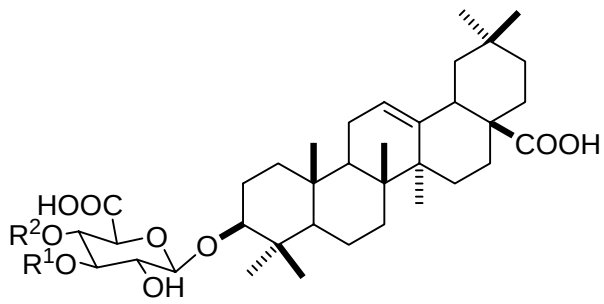
It has been found that hederagenin(**82**) had a marked inhibitory effect on the rate of protein biosynthesis in rat marrow (Das and Mahato, 1983).



82

2.2.6.6 Molluscicidal activity

Two saponins (**83**) and (**84**) showed molluscicidal activity against schistosomiasis-transmitting snails such as *Biomphalaria glabrata* (Borel et al.,1987).



83 R^1 = Rhm R^2 = H

84 R^1 = H R^2 = Glc

3 Aim of study

The main aim of this study is the isolation and structure determination of secondary metabolites from Cameroonian medicinal plants, to bring new elements into discussion relative to the relationship between the therapeutic properties of a plant used in traditional medicine and the natural products that plant may contain. For this purpose, crude extracts from plant materials will be separated using different chromatographic techniques such as thin layer chromatography (TLC), column chromatography (CC) and high performance liquid chromatography (HPLC). The structure of pure compounds obtained will be elucidated using different spectroscopic methods such as one-dimensional nuclear magnetic technique (^{1}H, ^{13}C NMR), and two-dimensional NMR techniques such as HMBC and HMQC. Each pure compound will be tested for biological activity.

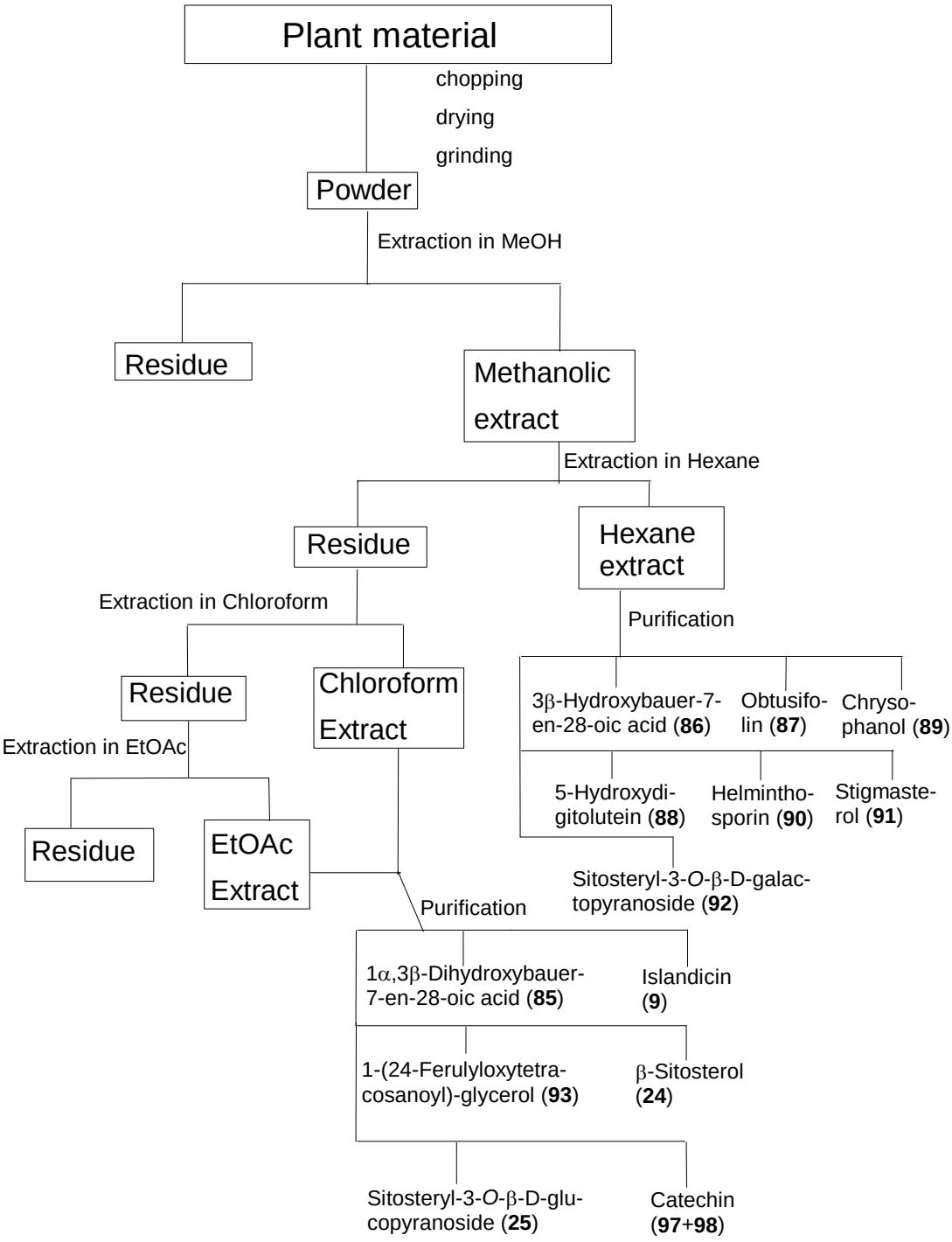
4 Results and discussion

4.1 Isolation and Characterisation of compounds from *Maesopsis eminii*

Air-dried stem bark of *Maesopsis eminii* was extracted with methanol at room temperature. The extract was concentrated to dryness to give a sticky residue. This residue was further re-extracted successively in petrol ether, chloroform and ethyl acetate. Chloroform and ethyl acetate extracts were combined on the basis of their similar composition (TLC), and the mixture was submitted to a column chromatography over silica gel followed by purification using different chromatographique techniques. A new triterpene, 1α,3β-dihydroxybauer-7-en-28-oic acid (**85**) in addition to known compounds 1-(24-ferulyloxytetracosnoyl)-glycerol (**93**), islandicin (**9**), β-sitosterol (**24**), sitosteryl-3 - *O* -β-D-glucopyranoside (**25**) and a mixture of (+)-catechin and (-)-epicatechin (**97**+**98**) were isolated from this fraction. The known triterpene 3β-hydroxy-bauer-7-en-28-oic acid (**86**) together with obtusifolin (**87**), chrysophanol (**89**), 5-hydroxydigitolutein (**88**) helminthosporin (**90**), stigmasterol (**91**), sitosteryl-3-*O*-β-D-galactopyranoside (**92**) were isolated from the petrol ether extract using different chromatographique techniques. Compound **93** was acetylated to its acetate derivative, **94**. Their structures have been elucidated by spectroscopic methods. The structure of the new triterpene (**85**) was confirmed by X-ray crystallographic analysis. This new triterpene displays moderate antibacterial activity against Gram-positive bacteria such as *bacillus subtilis ATCC* while **88** displays good anti-inflammatory activity (IC_{50} 9.5 μM) by inhibiting highly the 3α-hydroxysteroid dehydrogenase.

4.1.1 Extraction

Plant material was collected in the Centre Province of Cameroon, cut into pieces, air-dried and pulverized. The resulting powder has been extracted and purified chromatographically following the scheme below:



4.1.2 Identification of compounds

4.1.2.1 Identification of ME1

Compound **ME1** (**85**) was obtained as colorless crystals, mp 302-304 °C and reacted positively to the Liebermann-Burchard test for terpenoids. Its molecular formula $C_{30}H_{48}O_4$,

as established by ESI-FT-ICR mass spectrometry (*m/z* 471.34805; calcd. for $C_{30}H_{47}O_4$: 471.34798), corresponded to seven double bond equivalents.

The broad band decoupled ^{13}C NMR spectrum of compound **ME1** (**85**) showed 30 carbon signals which were assigned by DEPT and HMQC techniques as seven methyl groups, eight methylene groups, eight methine groups and seven quaternary carbons, among them a carbonyl group (δ 181.5), two oxygenated sp^3 carbons and two sp^2 carbons (see Table 4.1). The ^{13}C resonances at δ 144.9 and δ 115.3 were found to be characteristic of a Δ^7-bauerane skeleton (Mahato and Kundu, 1994).

The ^1H NMR spectrum of **ME1** (**85**) also contained resonances corresponding to seven methyl groups in the region δ 0.66 to δ 1.02, two of which appeared as doublets at δ 0.82 (*J* = 4.5 Hz) and δ 1.00 (*J* = 6.0 Hz). These were assigned to Me-29 and Me-30 respectively. The acid proton and the ethylenic proton (H-7), were observed as singlets at δ 12.07 (broad) and δ 5.35 respectively. The ^1H NMR spectrum also contained signals of two exchangable hydroxy protons at δ 4.35 and δ 4.19. The two hydroxy groups are attached to C-1 (δ 70.4) and C-3 (δ 70.8), respectively. All ^1H and ^{13}C signals were uniquely assigned by HH-COSY, HMQC, and HMBC experiments. Figure 4.1 shows the unique HMBC correlations of compound ME1 that provide proof for the connectivity and substitution pattern.

The CI mass spectrum of **ME1** (**85**) exhibited peaks at *m/z* 490 $[M+NH_4^+]$, 473 $[MH^+]$, 455 $[M\text{-}OH]^+$, the base peak at *m/z* 437 $[MH^+\text{-}2H_2O]$ and 427 $[M\text{-}COOH]^+$. The signal at *m/z* 263 is an indication for an oxygenated bauerene skeleton of which the base peak generally appears at *m/z* 231 (Budzikiewicz et al., 1963). This suggestion was further confirmed by the peak at *m/z* 227 corresponding to the loss of two water molecules from the fragment at *m/z* 263. The occurrence of these peaks can be explained by the fragmentation displayed in Figure 4.3.

The IR spectrum showed a strong and broad band at $\tilde{\nu}$ 3423 cm^{-1} (OH). The medium peak at $\tilde{\nu}$ 1380 cm^{-1} is indicative for two geminal methyl groups. In addition, a very strong and sharp band was observed at $\tilde{\nu}$ 1693 cm^{-1} (carboxyl). The presence of the trisubstituted ethylene function (C=CH-) in **ME1** (**85**) is supported by the multiple bands observed between $\tilde{\nu}$ 680 cm^{-1} and 1000 cm^{-1}. The structure obtained by assignment of the spectroscopic data was confirmed by the X-ray diffraction analysis of compound **ME1** (**85**). (see Figure 4.2 and Annex: Tables 7.1, 7.2, 7.3, 7.4, 7.5) . The relative configuration of the asymmetric carbon atoms was deduced from the X-ray data (Fokou et al., 2004). In

particular, in ring A of **ME1**, the hydroxy group on C-1 is axial and oriented below the plane of the molecule (1α-hydroxy) whereas the one at C-3 is equatorial, oriented above the plane (3β-hydroxy). Compound **ME1** is a new pentacyclic triterpene, named 1α,3β-dihydroxybauer-7-en-28-oic acid (**85**).

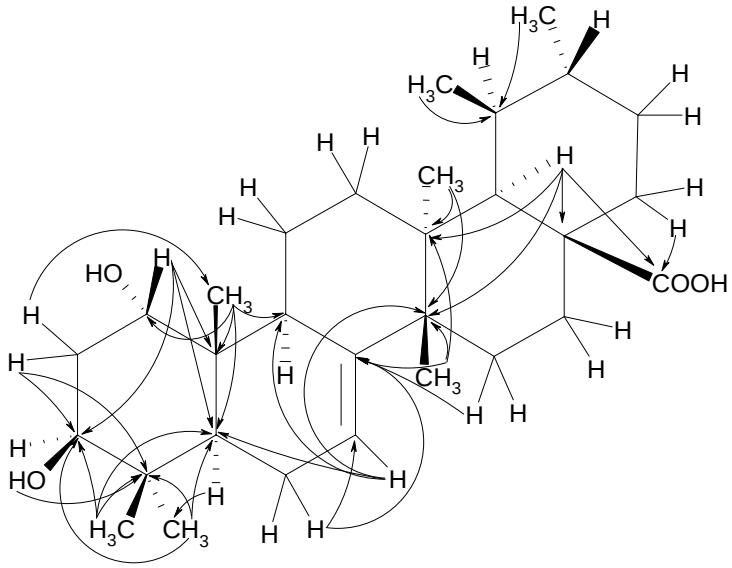


Figure 4.1: Selected HMBC correlations of compound **85**.

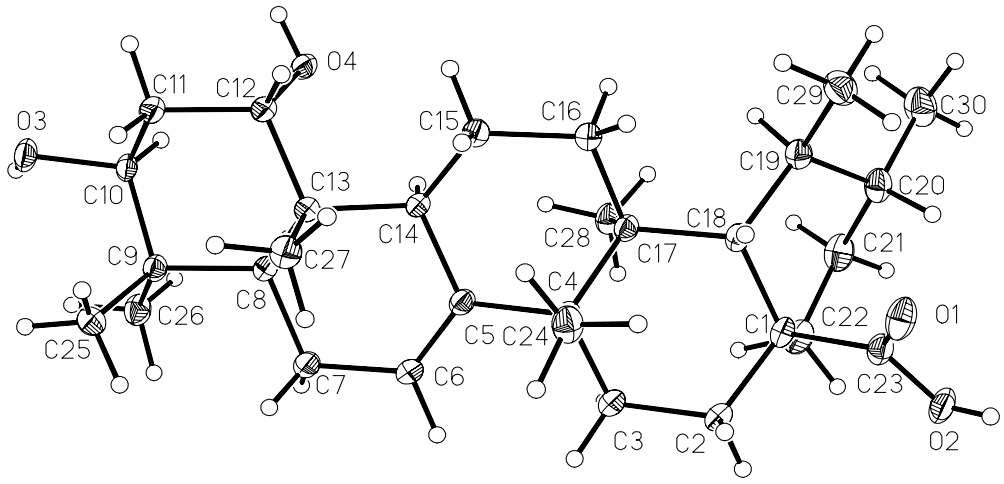
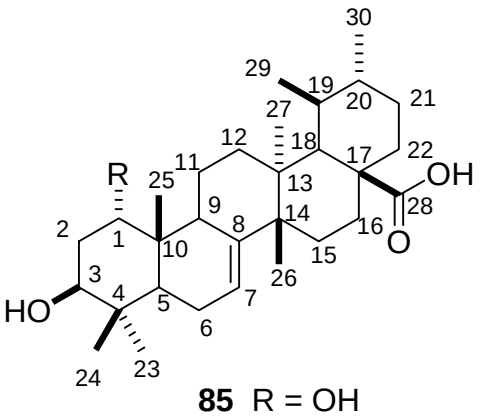


Figure 4.2: Molecular structure (relative configuration) of compound **85** according to X-ray analysis

4.1.2.2 Identification of ME2

Compound **ME2** (**86**) was isolated as a colorless powder and the molecular formula $C_{30}H_{48}O_3$ was established by EIMS (*m/z* 456 $[M]^+$). It gave positive response to the Liebermann - Burchard test for terpenoids. The ^1H NMR spectrum of **ME2** (**86**) was very similar to that of **ME1** (**85**), except that it exhibited the signal for only one hydroxy group at δ 4.32. The ^{13}C NMR contained resonances of 30 carbon atoms which were assigned after examination of the DEPT and HMQC spectra as seven methyl groups, nine methylene groups, seven methine groups and seven quaternary carbon atoms including the carbonyl group at δ 181.4. The signals at δ 144.2 and δ 116.4 lie in the same region as the carbons of the double bond in compound **ME1** (**85**) (δ 144.9 and δ 115.3) indicating that compound **ME2** (see Table 4.1 for ^{13}C NMR data) has the same carbon skeleton as compound **85**. This suggestion was also supported by the fragments at *m/z* 247 (63) and *m/z* 229 (66) present in the mass spectrum which were explained by the same fragmentation mechanism as for compound **ME1** (**85**). Additional fragments were observed at *m/z* 441 $[M\text{-}CH_3]^+$, 438 $[M\text{-}H_2O]^+$ and 423 $[M\text{-}H_2O\text{-}CH_3]^+$. As most naturally occurring polyoxygenated triterpenoids contain an oxygen function at position 3 (Gunasekera and Sutanbawa, 1977; Basu and Rastogi, 1967, Agarwal and Rastogi, 1974), the single hydroxy group was tentatively placed at that position. This was supported by the NMR data (Table 4.1). From its melting point, 308-310 °C, and its spectroscopic data compared to literature data (Mahato and Kundu, 1994; Chakravarty et al., 1991), to **ME2** was assigned the structure of 3β-hydroxy-bauer-7-en-oic acid (**86**), which melts at 305-308 °C (Meksuriyen et al., 1986).



85 R = OH
86 R = H

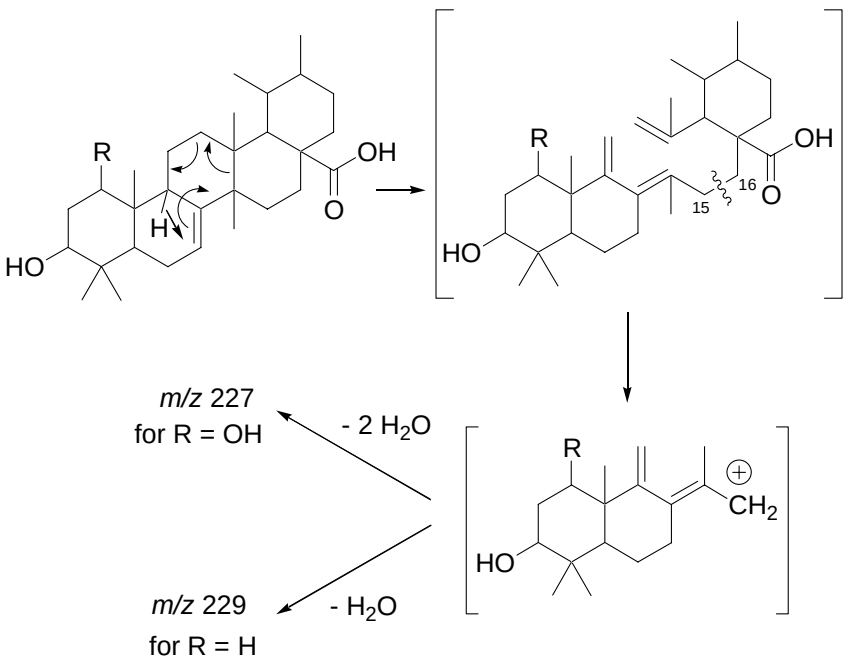


Figure 4.3: Fragmentation mechanism of **ME1** (**85**) and **ME2** (**86**)

Table 4.1: NMR Data of **ME1** (**85**) and **ME2** (**86**), recorded in DMSO-D_6

	85		**86**
Atom No	^{1}H NMR	^{13}C NMR	^{13}C NMR
1	3.50; s	70.4; CH	36.3; CH_2
2	1.62; 1.67; m	35.0; CH_2	27.4; CH_2
3	3.59; d	70.8; CH	76.8; CH
4		38.6; C	38.4; C
5	1.57; s	43.2; CH	49.9; CH
6	1.92; m 2.08; m	23.8; CH_2	23.7; CH_2
7	5.35; s	115.3; CH	116.4; CH
8		144.9; C	144.2; C
9	1.60; m	43.2; CH	47.2; CH
10		38.5; C	34.7; C
11	1.50; m	27.8; CH_2	27.6; CH_2
12	1.52; m	32.1; CH_2	32.0; CH_2
13		36.6; C	36.6; C
14		40.6; C	40.5; C
15	1.43; m 1.73; m	15.0; CH_2	15.8; CH_2
16	1.30; m 1.75; m	32.1; CH_2	32.0; CH_2
17		43.8; C	43.7; C
18	2.31; s	47.6; CH	47.5; CH
19	1.05	31.6; CH	31.6; CH
20	1.10	36.2; CH	36.1; CH
21	1.07; m 1.54; m	28.8; CH_2	28.7; CH_2
22	1.60; m 2.15; m	25.5; CH_2	25.5; CH_2
23	0.75; s	14.8; CH_3	14.9; CH_3
24	0.87; s	27.8; CH_3	27.6; CH_3
25	0.66; s	13.1; CH_3	12.6; CH_3
26	1.02; s	23.1; CH_3	23.1; CH_3
27	0.98; s	21.2; CH_3	21.3; CH_3
28		181.5; C	181.4; C
29	0.82; d	21.1; CH_3	21.1; CH_3
30	1.00; d	23.3; CH_3	23.2; CH_3

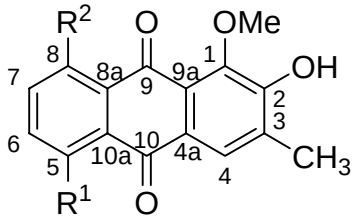
4.1.2.3 Identification of ME3

This compound has been obtained as a yellow powder, mp 239-241 °C. The ESI mass spectrum (positive mode) showed a peak at *m/z* 590.8 $[2M+Na]^+$ and in negative mode a peak at *m/z* 282.9 $[M-H]^-$. Its molecular formula $C_{16}H_{12}O_5$, as established by ESI-FT-ICR mass spectrometry (*m/z* 285.07539; calcd. for $C_{16}H_{13}O_5$: 285.07575), corresponded to 11 double bond equivalents.

In the ^1H NMR spectrum (500 MHz), a singlet at δ 12.83 for a chelated hydroxyl group (8-OH) and four signals for aromatic protons at δ 7.98 (s); 7.78 (1H, d, *J* = 6.9 Hz, H-5); 7.63 (1H, t, *J* = 7.8 Hz, H-6); 7.27 (1H, d, *J* = 7.8 Hz, H-7) were observed. Another singlet at δ 6.76 was exchangeable in D_2O and was assigned to 2-OH. Except the singlet at δ 7.98, the three other aromatic protons formed an ABC system according to their coupling constants. Two more signals appeared in the aliphatic region of the proton spectrum at δ 4.00 and 2.40 and were attributed to a methoxy and an aromatic methyl groups respectively.

The ^{13}C NMR spectrum contained resonances of 16 carbon atoms which were assigned after examination of the DEPT and HMQC spectra as one methyl group, one methoxy group (δ 62.29), four methine groups and 10 quaternary carbon atoms including two carbonyl groups at δ 188.67 (C-9) and δ 181.72 (C-10). The signal of the carbonyl group at δ 188.7 confirmed the presence of a chelated hydroxy group, and thus suggested the anthraquinone skeleton. The complete ^{13}C NMR data are reported on Table 4.2. The proton and carbon assignment is based on the DEPT and HMQC spectra. The different interactions observed in the the HMBC spectrum supported the assignment. In fact, the ^1H NMR singlet at δ 7.98 (H-4) gave cross peaks with the carbonyl group at δ 181.72 (C-10), one quaternary carbon atom (δ 123.08, C-9a), the aromatic methyl group at δ 16.43 (3-Me) and the aromatic carbon with *O*-function at δ 153.90 (C-2). One of the ABC system protons, which appeared at δ 7.78 (H-5) gave cross peaks with the carbonyl group at δ 181.72 (C-10), one quaternary carbon atom (δ 116.82, C-8a) and one tertiary CH aromatic carbon at δ 124.22 (C-7). The proton triplet at δ 7.63 (H-6) gave cross peaks with two quaternary carbon atoms at δ 132.24 (C-10a) and δ 162.44 (C-8) respectively. These results proved that the chelated hydroxyl group was located at C-8. Hence the aromatic protons which showed a doublet signal at δ 7.63 and a triplet signal at δ 7.27 were attributed to H-7 and H-6, respectively. The above results were also supported by HH-COSY spectrum.

Finally **ME3** was characterised as 2,8-dihydroxy-1-methoxy-3-methylanthraquinone (**87**), known as obtusifolin which melts at 241-243 °C (Cameron et al.,1989).



87 $R^1 = H$ $R^2 = OH$

88 $R^1 = OH$ $R^2 = H$

4.1.2.4 Identification of ME4

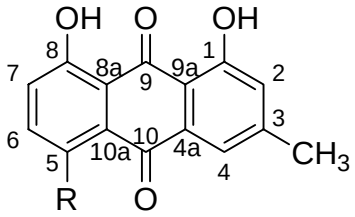
ME4 presents a cotton wool aspect with a yellow colour, mp 171-174 °C. Its CI mass spectrum show peaks at *m/z* 255 $[MH]^+$ and 254 $[M]^+$. Its molecular formula, $C_{15}H_{10}O_4$, was established by HRMS (CI) (*m/z* 255.06549; calcd. for $C_{15}H_{11}O_4$: 255.06573) and corresponded to 11 double bonds equivalents.

The 500 MHz ^{1}H NMR spectrum showed two singlets for the chelated hydroxyl groups at δ 12.10 and 11.99; an ABC system at δ 7.80 (1H, dd, *J* = 7.5, 1.0 Hz, H-5), δ 7.66 (1H, dd, *J* = 8.0, 7.5 Hz, H-6) and δ 7.28 (1H, dd, J = 8.00, 1.0 Hz, H-7), which suggest a 1,2,3-trisubstituted aromatic ring; one aromatic methyl proton at δ 2.46 and two further aromatic protons at δ 7.08 (1H, d, *J* = 1.2 Hz) and 7.63 (1H, d, *J* = 1.2 Hz), suggesting a a 1,3,5,6-tetrasubstituted aromatic ring. All these informations also suggest an anthraquinone skeleton.

Examination of the complete ^{13}C NMR and DEPT spectra reveals the presence of 15 carbon atoms which were sorted as one methyl group at δ 22.3 , five methine groups and nine quaternary carbon atoms including two carbonyl groups (see Table 4.2 for complete ^{13}C NMR data). These data were found to be similar to those reported for chrysophanol (Knut at al., 1992).

The IR spectrum showed peaks at $\tilde{\nu}$ 3429, 2921, 2851, 1672, 1627, 1457, 1384, 1272, 1210, 1055, 753 and 727 cm^{-1}. The 1672 and 1627 cm^{-1} bands can be assigned to an unchelated carbonyl and a chelated carbonyl group in anthraquinone (Joshi et al., 1962).

From all these informations, **ME4** was therefore characterised as 1,8-dihydroxy-3-methylanthraquinone (**89**), known as Chrysophanol (Cumming and Thomson, 1970; Dagne and Steglich, 1984; Knut at al., 1992).



89 R = H

90 R = OH

4.1.2.5 Identification of ME5

ME5 crystallises as orange needles, mp 235 – 237 °C. Its CI mass spectrum presented peaks at *m/z* 285 $[MH]^+$, 284 $[M]^+$. Additional peaks appear at *m/z* 271, 266, 255, 238. Its molecular formula $C_{16}H_{12}O_5$, was established by ESI-FT-ICR mass spectrometry (*m/z* 285.07554 $[MH]^+$; calcd. for $C_{16}H_{13}O_5$: 285.07575 $[MH]^+$), and corresponded to 11 double bond equivalents.

The ^1H NMR spectrum presented two signals for one aromatic methyl and one *O*-methyl groups respectively at δ 2.36 and 3.93; a signal attributed to a chelated hydroxyl group appeared at δ 12.93. Signals of three aromatic protons giving an ABC system at δ 7.34 (1H, dd, *J* = 7.5, 1.3 Hz, H-6); δ 7.79 (1H, t, *J* = 7.9 Hz) and δ 7.68 (1H, dd, *J* = 7.5, 1.3 Hz, H-8) and a one proton singlet at δ 7.86 (1H, s, H-4) were observed in the aromatic region.

The ^{13}C NMR spectrum of **ME5** also contained resonances of 16 carbon atoms which were assigned after examination of the DEPT and HMQC spectra as one methyl group, one methoxy group (δ 61.71), four methine groups and 10 quaternary carbon atoms including two carbonyl groups at δ 181.77 (C-9) and δ 189.50 (C-10) (see Table 4.2 for complete ^{13}C NMR data).

A HMBC NMR experiment was employed to determine the position of the chelated hydroxyl group. The proton singlet at δ 7.86 was assigned to C-4 based on the HMBC correlations of H-4 to C-10 (three bonds), H-4 to C-9a (three bonds), H-4 to C-9 (four bonds). The methyl group was located at C-3 according to the HMBC correlations of its

protons to C-4 (three bonds), C-2 (three bonds) and C-4a (four bonds). The HMBC correlation from C-10 to the chelated OH proton (three bonds) indicated the location of this chelated OH group at C-5. One of the ABC system protons, which appeared at δ 7.34 (H-6) gave cross peaks with the carbonyl group at δ 189.50 (C-10), one quaternary carbon atom (δ 133.72, C-10a) and one aromatic carbon with *O*-function at δ 163.20 (C-5). The two remaining aromatic protons were terefore located at C-7 and C-8.

The IR spectrum showed peaks at $\tilde{\nu}$ 3333 (OH), 1633, 1560, 1475, 1315, 1272, 1092, 1038 and 749 cm^{-1}.

All these spectroscopic data together with the meleting point (Lit. 234 – 236 °C; Imre, 1973) have been found to be close to those of 5-hydroxydigitolutein (**88**) isolated and described for the first time by Imre in 1973. Therefore **ME5** was 5-hydroxydigitolutein.

4.1.2.6 Identification of ME6

ME6 crystallised as a red powder, mp 196-198 °C. It CI mass spectrum presented a peak at *m/z* 271 $[MH]^+$, 255 $[MH\text{-}O]^+$. Its molecular formula, $C_{15}H_{10}O_5$, as established by HRMS (CI) (*m/z* 271.06008 $[MH]^+$; calcd. for $C_{15}H_{11}O_5$: 271.06065) corresponded to 11 double bonds equivalent.

The 500 MHz ^1H NMR spectrum presented three singlets at δ 12.97, 12.28 and 12.10 attributed to the chelated hydroxyl groups; one multiplet of two protons at δ 7.24 (2H, H-6, H-7) and two other aromatic protons at δ 7.66 and 7.08. A signal of an aromatic methyl group was observed at δ 2.46. The presence of an anthraquinone skeleton was strongly supported by the three chelated hydroxyl groups.

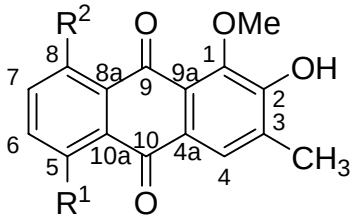
The broad band decoupled ^{13}C NMR spectrum of **ME6** showed 15 carbon signals which were assigned by DEPT and HMQC techniques as four methine groups (δ 129.6, 129.5, 124.4 and 121.0), 10 quaternary carbons, among them two carbonyl groups (δ 190.58 and δ 186.57) and one methyl group (δ 22.27) (see Table 4.2 for complete ^{13}C NMR data).

The signals at δ 129.6 and 129.5 together with the one observed in the ^1H NMR spectrum at δ 7.24 integrating for two protons demonstrated that the two protons are in the same chemical environment. These two protons thus assigned to C-6 and C-7.

The methyl group was located at C-3 according to the HMBC correlations of its protons to C-4 (three bonds) and C-2 (three bonds) and the HMBC correlations of proton H-4 to C-2 and the carbon of the methyl group.

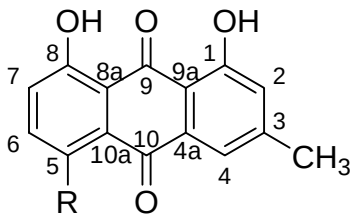
The IR spectrum showed peaks at $\tilde{\nu}$ 3433 (OH), 1602, 1457, 1296, 1260, 1231, 1181 and 805 cm^{-1}.

From all these informations, **ME6** was identified as 1,5,8-trihydroxy-3-methylanthraquinone, known as Helminthosporin (**90**) (Barba et al., 1992).



87 R^1 = H R^2 = OH

88 R^1 = OH R^2 = H



89 R = H

90 R = OH

Table 4.2: ^{13}C-NMR data of compounds (**87**), (**88**), (**89**), (**89**[a]) and (**90**)

	87	**88**	**89**	**89**[a]	**90**
Atom no.	^{13}C-NMR ($CDCl_3$)	^{13}C-NMR ($CDCl_3$)	^{13}C-NMR ($CDCl_3$)	^{13}C-NMR (DMSO)	^{13}C-NMR ($CDCl_3$)
1	146.25; C	148.23; C	162.40; C	161.67	162.80; C
2	153.05; C	156.75; C	124.36; CH	124.16	124.40; CH
3	133.05; C	133.63; C	149.30; C	149.26	149.10; C
4	126.98; CH	126.68; CH	121.36; CH	120.64	120.80; CH
4a	126.46; C	126.00; C	133.26; C	133.10	133.26; C
5	119.03; CH	163.20; C	119.90; CH	119.41	157.56; C
6	136.35; CH	124.41; CH	136.95; CH	137.41	129.50; CH
7	124.22; CH	137.18; CH	124.56; CH	124.49	129.60; CH
8	162.44; C	118.88; CH	162.70; C	161.41	133.26; C
8a	116.82; C	117.52; C	115.86; C	115.94	112.48; C
9	188.67; C	181.77; C	192.50; C	191.72	190.58; C
9a	123.08; C	124.47; C	113.70; C	113.85	113.93; C
10	181.72; C	189.50; C	181.97; C	181.57	186.57; C
10a	132.24; C	133.72; C	133.60; C	133.40	136.95; C
Me	16.43	16.81	22.27	21.71	22.27
MeO	62.29	61.71			

[a] ^{13}C-NMR (DMSO) from literature (Knut et al., 1992)

4.1.2.7 Identification of ME7

ME7 crystallised from a mixture of PE-EtOAc 7:3 to give a colorless powder, mp 138-140 °C. This powder was easily soluble in chloroform and gave a positive response to the Liebermann-Burchard test for steroid.

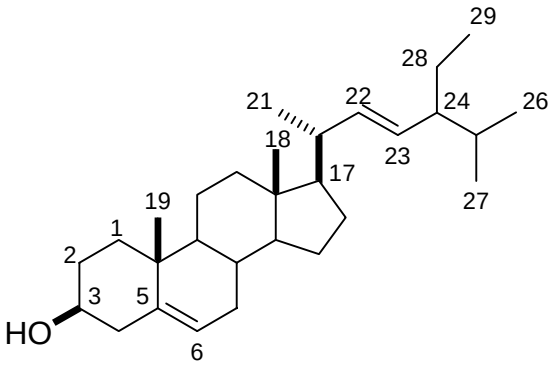
The CI mass spectrum showed peaks at *m/z* 413 $[MH]^+$ and 395 $[M\text{-}OH]^+$. Its molecular formula $C_{29}H_{48}O$, was established by HRMS (EI) (*m/z* found: 412.37047 $[M]^+$; calcd.: 412.37052 $[M^+]$), and corresponded to six double bond equivalents. The steroid skeleton was also confirmed by the peaks at *m/z* 273, 271, 255 which were explained by the same fragmentation mechanism as for β-sitosterol displayed in figure 4.6.

The 500 MHz ^{1}H NMR spectrum of this compound showed the presence of three olefinic protons at δ 5.33 (1H, s br, H-6), 5.12 (1H, dd, *J* = 15.1, 8.8 Hz, H-22), 5.10 (1H, dd, *J* = 15.1, 8.8 Hz, H-23) respectively. The coupling constants of latter two signals correspond to *E*-configuration. The presence of a steroid skeleton was further confirmed by the ^{13}C NMR signals at δ 129.2 and 138.3, characteristic of Δ^{22}–sterol (Rubinstein et al., 1976), and δ 140.7 and 121.7 characteristic of Δ^5–sterol skeleton (Tandon et al., 1990). In addition, the ^{1}H NMR spectrum showed a multiplet at δ 3.55 corresponding to the proton H-3 and signals of six methyl groups in the region δ 0.53 to δ 1.02.

The ^{13}C NMR contained resonances of 29 carbon atoms which were assigned by DEPT and HMQC techniques as six methyl groups in the region δ 11.80 to δ 20.00, nine methylene groups, 11 methine groups of which one (δ 71.8) was oxygenated and three quaternary carbon atoms at δ 140.7, 42.3 and 36.5 (see Table 4.4 for complete ^{13}C NMR data). The oxygenated carbon atom was deduced to be C-3 based on biosynthetic arguments.

The IR spectrum showed a broad band at $\tilde{\nu}$ 3416 cm^{-1} (OH). Additional peaks were observed at $\tilde{\nu}$ 2865, 1463, 1380, 1055, 1022, 958, 838 and 800 cm^{-1}.

From all those physical and spectroscopic data together with the literature, **ME7** was identified to stigmasterol (**91**) already described by Ikan and which melts at 139-140 °C (Ikan, 1991b).



91

4.1.2.8 Identification of ME8

ME8 crystallised from a mixture of CH_2Cl_2-MeOH 20:1 to give a colorless powder, mp 261-263°C. It gave a positive response to the Liebermann-Burchard test for steroids and also a blue-violet colour when heated with 10% H_2SO_4 in MeOH after TLC. The ESI mass spectrum showed a quasimolecular ion at *m/z* 599 $[M+Na]^+$, which suggested a molecular mass of 576.

The EI mass spectrum displayed fragments at *m/z* 414 $[M_{aglycone}]^+$ and *m/z* 396 due to the loss of a hexose, presumably glucose from the molecular ion peak (see Figure 4.4). This lead to the molecular formula $C_{35}H_{60}O_6$.

The 500 MHz ^{1}H NMR spectrum of this compound showed the following signals: an olefinic proton (H-6) at δ 5.31 ; six methyl groups in the region δ 0.64 to δ 0.95 [δ 0.64 (3H, s, Me-18); 0.95 (3H, s, Me-19); 0.89 (3H, d, *J* = 6.5, Me-21); 0.80 (6H, d, *J* = 6.9, Me-26, Me-27); 0.81 (3H, t, *J* = 6.5, Me-29)]. These were found to be typical signals for sitosterol (Iribarren and Pomicio, 1984). In addition to the common signals of a sitosterol asignal at δ 4.20 (d, *J* = 7.8 Hz) was observed which was assigned to an anomeric proton (H-1'). The coupling constant ^3J was characteristic for an axial-axial coupling, thus showing that the sugar moiety was β-linked to the aglycone (Alam et al., 1996). Further signals resonating at δ 2.88, 3.00, 3.1, 3.30, 3.40 and 3.62 appeared as multiplet. The signals at δ 4.86 (1H, d, *J* = 4.7 Hz), 4.83 (2H, t, *J* = 5.7 Hz) and 4.40 (1H, *J* = 7.5 Hz) were attributed to four protons exchangeable with D_2O due to four free hydroxy groups of the sugar moiety.

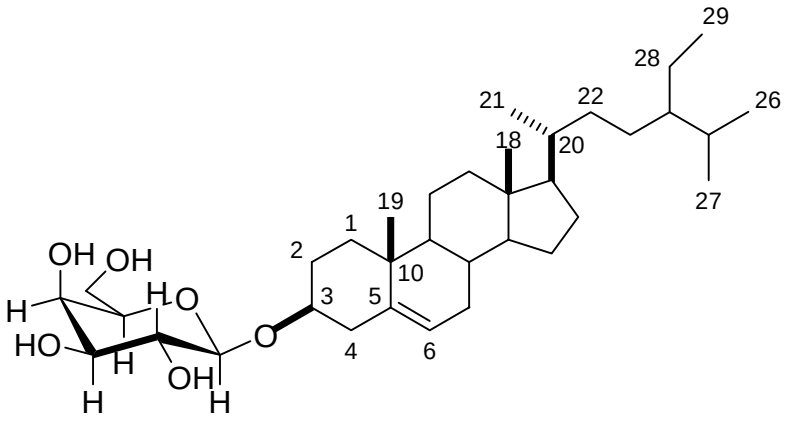
The ^{13}C NMR spectrum showed the presence of 35 carbon atoms in the molecule. The number of hydrogen substituents of each carbon atom was determined by DEPT and HMQC experiments. Examination of the ^{13}C NMR and DEPT NMR spectra revealed the

presence of six methyl groups, 12 methylene, 14 methine, and three quaternary carbon atoms (see Table 4.4 for complete ^{13}C NMR data). Particular signals at δ 100.7 (C-1'), 76.7 (C-3' and C-5'), 73.4 (C-2'), 70.0 (C-4') and 61.0 (C-6') indicated the presence of a single monosaccharide moiety. These signals were in agreement with those obtained from the literature (Agrawal, 1992; Jahan et al., 1995) for the β-D-Galactose. All ^{1}H and ^{13}C signals were uniquely assigned by HH-COSY, HMQC and HMBC experiments. The HH-COSY spectrum showed a strong coupling interaction between the proton H-3 of the methine group and proton H-4 of the methylene group.

Acid hydrolysis of **ME8** yielded D-galactose and the aglycone which was identified as β-sitosterol (**24**) by spectral data as well as by direct comparison with the authentic sample (TLC, R_f 0.42 in $CHCl_3$/MeOH 97:3).

The IR spectrum showed a broad band at $\tilde{\nu}$ 3400 cm^{-1} (OH). Additional peaks were observed at $\tilde{\nu}$ 3351, 3000, 2853, 1645, 1375, 1350, 1168, 1070, 1030 cm^{-1}.

From all these informations together with the literature, **ME8** was identified as sitosterol 3-*O*-β-D-galactopyranoside (**92**).



92

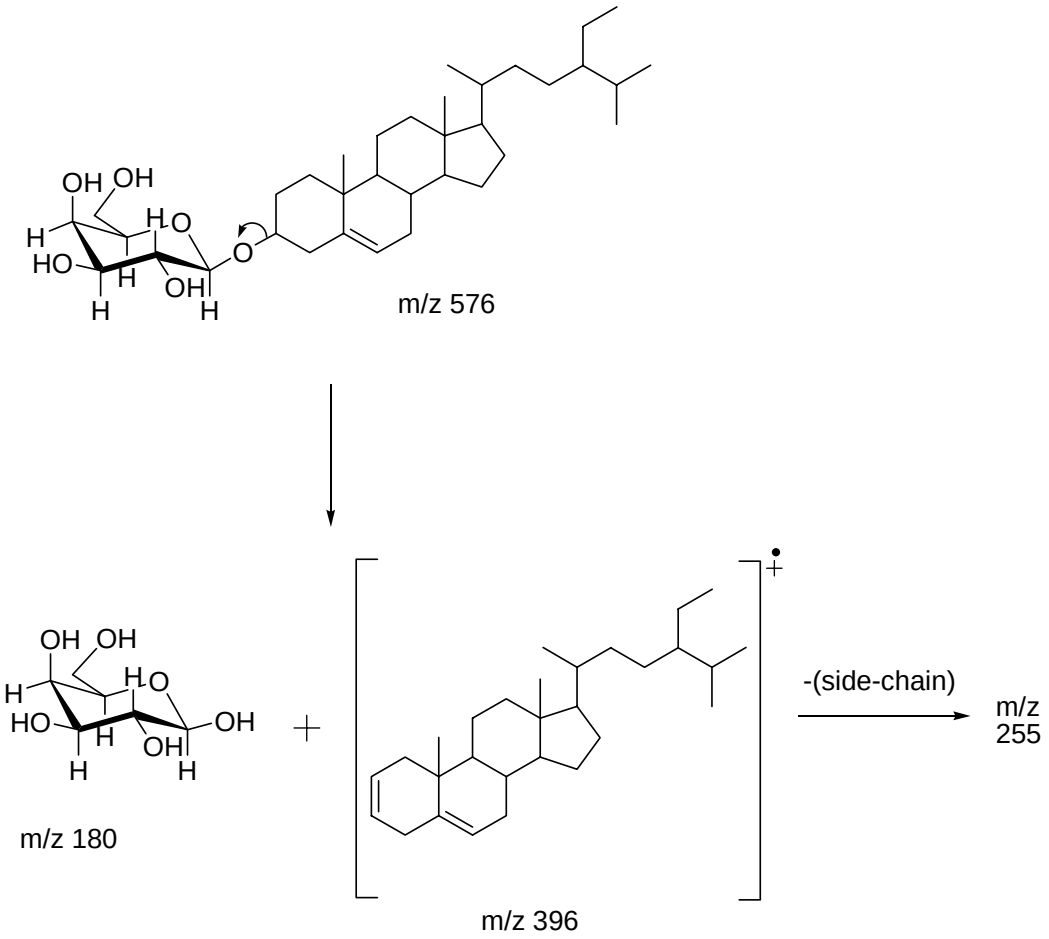


Figure 4.4: Fragmentation mechanism of Δ^5–sterol glucosides

4.1.2.9 Identification of ME9

ME9 crystallised as a brown powder in chloroform, mp 74-76 °C. Its ^{1}H NMR spectrum showed that it was composed of three moieties. One was trans-4-hydroxy-3-methoxycinnamate, with aromatic protons at δ 6.90 (d, *J* = 8.2 Hz), δ 7.03 (d, *J* = 1.9 Hz) and δ 7.06 (dd, *J* = 8.2, 1.9 Hz); a methoxy group at δ 3.91 and a trans-configured double bond: δ 6.29 (d, *J* = 15.9 Hz) and δ 7.60 (d, *J* = 15.9 Hz). The second moiety showed two distinctive methylene groups connected to an oxycarbonyl group (δ 4.18, t, *J* = 6.8 Hz) and a carbonyloxy group (δ 2.33, t, *J* = 7.5 Hz); two further methylene units connected to these methylene groups mentioned above (δ 1.7, t, *J* = 6.8 Hz; δ 1.6, m, *J* = 7.2 Hz); one more methylene group (δ 1.37, m) together with a serie of methylene groups as a long aliphatic chain (36H, δ 1.22-1.27, br s). The third moiety was identified as a glycerol δ 3.91 (1H, m), δ 4.15 (2H, d, *J* = 6.0 Hz), and δ 3.58 (dd, *J* = 11.3, 6.0 Hz) and 3.69 (1H, dd, *J* = 11.5,

3.9 Hz). The glycerol moiety was monosubstituted on position 1, since only one methylene group of this glycerol was shifted downfield δ 4.15 (2H). This suggestion was confirmed after acetylation of **ME9**. The ^{1}H NMR spectrum of its acetate derivative showed three acetyl groups; one for the phenol (δ 2.33) and two for the aliphatic alcohols (δ 2.06 and δ 2.07).

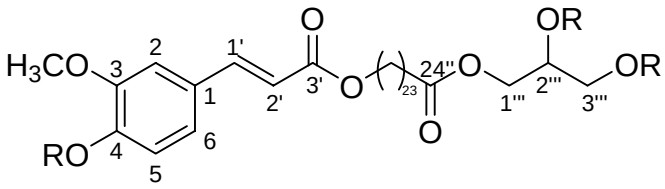
The ESI mass spectrum (positive mode) of **ME9** gave a pseudomolecular ion peak at *m/z* 657.5 $[M+Na]^{+}$. Particular peaks were observed on this ESI-MS at *m/z* 634.4 $[M]^{+}$, corresponding to the molecular formula $C_{37}H_{62}O_{8}$, having seven double bond equivalents; and *m/z* 559.4 $[M-C_{3}H_{7}O_{2}]^{+}$. Additional peaks were also observed at *m/z* 541.4, 473.4, 177. The CI mass spectrum showed the base peak at *m/z* 298, and other signals at *m/z* 543 $[M-C_{3}H_{7}O_{3}]^{+}$, 542, 498, 296, 242 and 177. The fragmentation mechanism displayed in figure 4.5 gave an explanation on the occurrence of the important peaks. The ESI mass spectrum of the acetate derivative obtained after acetylation of **ME9** showed the molecular ion peak at 761.3 $[MH]^{+}$ leading to the molecular formula $C_{43}H_{68}O_{11,}$ DBE = 10, confirming that the phenol and the two aliphatic alcohols were acetylated.

The ^{13}C NMR spectrum of **ME9** showed a series of carbon signals which were sorted by DEPT and HMQC techniques as five quaternary carbons including two carbonyl groups at δ 174.39 and δ 167.50, six methine groups of which one (δ 70.22) was oxygenated, one methoxy group (δ 55.92), seven methylene groups of which three were oxygenated, and additional methylene groups appearing as a long and broad signal (δ 29.69 – 29.14) attributed to a long aliphatic chain.

The IR spectrum showed a broad band at $\tilde{\nu}$ 3422 cm^{-1} (OH). Additional peaks were observed at $\tilde{\nu}$ 2917, 2849, 1737, 1712, 1632, 1596, 1518, 1469, 1273, 1174 and 719 cm^{-1}.

The ^{1}H NMR data of **ME9** showed similarities with those obtained from the literature for a mixture of $C_{22\text{-}28}$ ω-hydroxy acid esters of cis and trans-ferulic acid (Kawanishi and Hashimoto, 1987).

From all these findings, together with the literature, we assigned to **ME9** the structure bellow, which correspond to 1-(24-ferulyloxytetracosanoyl)-glycerol (**93**).



93 R = H

94 R = Ac

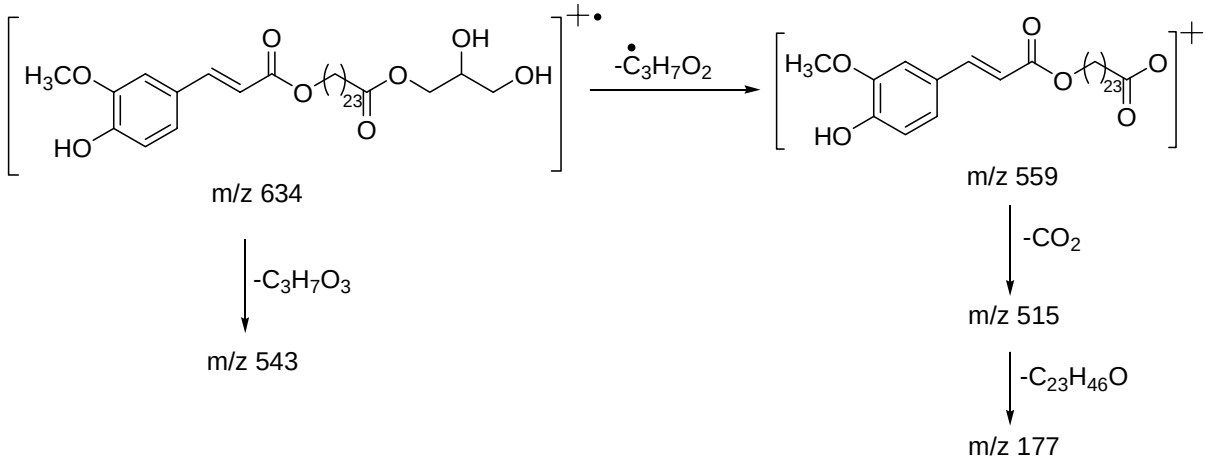


Figure 4.5: Fragmentation mechanism of **ME9**

4.1.2.10 Identification of ME11

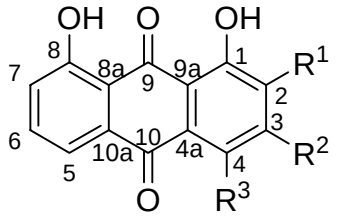
ME11 was obtained as red brown powder (6 mg), mp 215-217 °C, soluble in chloroform. The CI mass spectrum showed peaks at *m/z* 271 $[MH]^+$ and 270 $[M]^+$, Its molecular formula, $C_{15}H_{10}O_5$, as established by HRMS (EI) (*m/z* 270.05322; calcd. for $C_{15}H_{10}O_5$: 270.05282) corresponded to 11 double bond equivalents.

The 500 MHz ^{1}H NMR spectrum presented three singlets at δ 13.48, 12.37 and 12.32 due to three chelated hydroxyl groups; three protons giving an ABC system at δ 7.88 (d, 1H, *J* = 7.6 Hz), 7.68 (t, 1H, *J* = 7.6 Hz) and 7.29 (d, 1H, *J* = 7.6 Hz). In addition, signals of one aromatic proton singlet at δ 7.15 and one aromatic methyl group at δ 2.37 were observed on this spectrum. The three chelated hydroxyl groups strongly suggested an anthraquinone skeleton.

The broad band decoupled ^{13}C NMR spectrum of **ME11** showed 15 carbon signals which were assigned by DEPT and HMQC techniques as four methine groups at δ 136.69 (C-6),

129.04 (C-2), 124.52 (C-7) and 119.38 (C-5) and 11 quaternary carbons, among them two carbonyl groups at δ 190.45 (C-9) and δ 186.56 (C-10).

According to these data, two structures (**9**, **95**) were possible. The ^{1}H NMR data of **ME11** were identical to those reported in the literature for islandicin (**9**) (Simoneau and Brassard, 1988; Jammula et al., 1991). In addition its ^{1}H and ^{13}C NMR spectra showed similarities to those reported for islandicin 4-methyl ether (**96**) except that the signal of the third chelated hydroxyl group was replaced by a signal of a methoxy group located at C-4 (Lin et al., 2001). The compared data are reported in Table 4.3. A HMBC NMR experiment was also employed to determine the position of the chelated hydroxyl groups and the aromatic methyl group. In fact the chelated OH proton at δ 13.48 (4-OH) was correlated with the signal at δ 157.88 (C-4), δ 111.66 (C-4a), δ 141.81(C-3). In turn the signal at δ 157.88 (C-4) was correlated with resonances at δ 2.37 (Me-3) and δ 7.15 (H-2); the signal at δ 129.04 (C-2) correlated with resonances at δ 2.37 (Me-3) and δ 12.37 (1-OH). The proton singlet (δ 7.15) also showed a cross peak with the carbonyl group at δ 190.45 (C-9), which was possible if the proton was attached to C-2. Therefore, **ME11** was identified as islandicin (**9**) which melts at 218.5-219 °C (Simoneau and Brassard, 1988).



9 $R^1 = H$ $R^2 = CH_3$ $R^3 = OH$

95 $R^1 = CH_3$ $R^2 = H$ $R^3 = OH$

96 $R^1 = H$ $R^2 = CH_3$ $R^3 = OCH_3$

Table 4.3: NMR data of **ME11** (**9**) and its 4-methyl ether (**96**)

	96		**9**	
Atom no.	^{1}H-NMR ($CDCl_3$)	^{13}C-NMR ($CDCl_3$)	^{1}H-NMR ($CDCl_3$)	^{13}C-NMR ($CDCl_3$)
1		159.6; C		157.70; C
2	7.18, s	126.9; CH	7.15, s	129.04; CH
3		146.6; C		141.81; C
4		153.8; C		157.88; C
4a		123.6; C		111.66; C
5	7.81, d, (7.5 Hz)	120.0; CH	7.88, d, (7.6 Hz)	119.38; CH
6	7.68, t, (7.5 Hz)	137.1; CH	7.68, t, (7.6 Hz)	136.69; CH
7	7.27, d, (7.5 Hz)	123.6; CH	7.29, d, (7.6 Hz)	124.52; CH
8		162.0; C		162.53; C
8a		115.5; C		116.25; C
9		192.3; C		190.45; C
9a		114.0; C		110.71; C
10		181.4; C		186.56; C
10a		134.8; C		133.59; C
Me	2.41, s	17.2	2.37, s	16.63
MeO	3.89, s	61.0		
1-OH	12.51, s		12.37, s	
4-OH			13.48, s	
8-OH	12.06, s		12.32, s	

4.1.2.11 Identification of ME12

ME12 crystallised from a mixture of PE-$CHCl_3$ 1:1 to give colorless powder, mp 140-141 °C. This powder was easily soluble in chloroform and gave a positive response to the Liebermann-Burchard test for steroids.

The CI mass spectrum showed peaks at *m/z* 432 $[M+NH_4]^+$ 414 $[M]^+$and 396 $[M-H_2O]^+$, giving rise to the molecular formula $C_{29}H_{50}O$, as established by HRMS (EI) (*m/z* found: 414.38581 $[M]^+$; calcd.: 414.38617 $[M]^+$), and corresponded to five double bond

equivalents. The steroid skeleton was also confirmed like in **ME7** by the peaks at *m/z* 273, 271, 255 which were explained by the fragmentation mechanism displayed in figure 4.6.

The ^{1}H NMR spectrum of **ME12** was very similar to that of **ME7**, except that it exhibited the signal for only one olefinic proton at δ 5.30 (1H, s br, H-6). The ^{13}C NMR contains also resonances of 29 carbon atoms which were assigned by DEPT and HMQC techniques as six methyl groups, 11 methylene groups, nine methine groups of which one (δ 70.5, C-3) was oxygenated and three quaternary carbon atoms (see Table 4.4 for complete ^{13}C NMR data). **ME12** was identified as β-sitosterol (**24**) by direct comparison of its mp (Lit. 139-140 °C; Ikan, 1991b) and NMR data to those obtained from the literature (Ikan, 1991b; Koizumi et al., 1979).

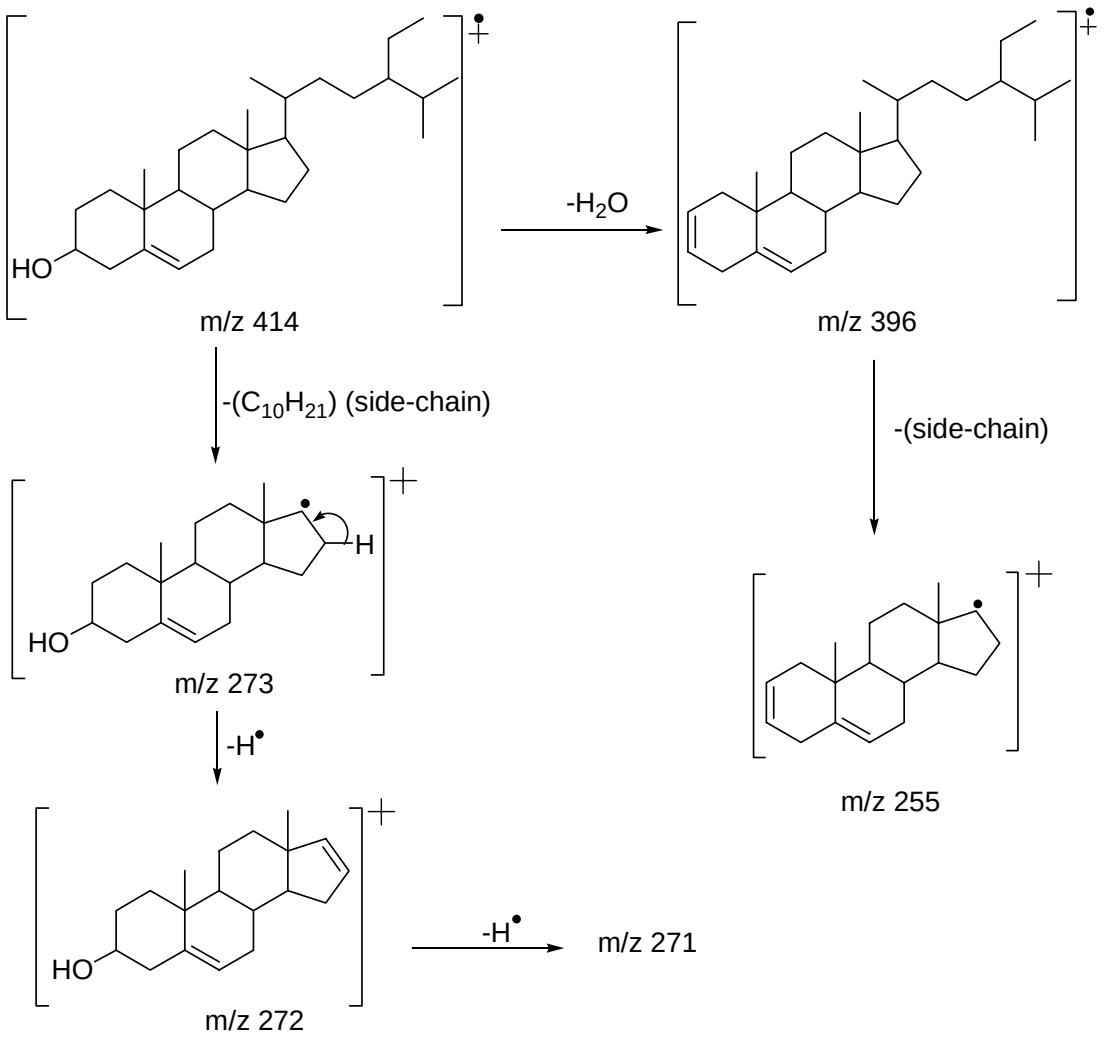


Figure 4.6: Fragmentation mechanism of β-sitosterol (**24**)

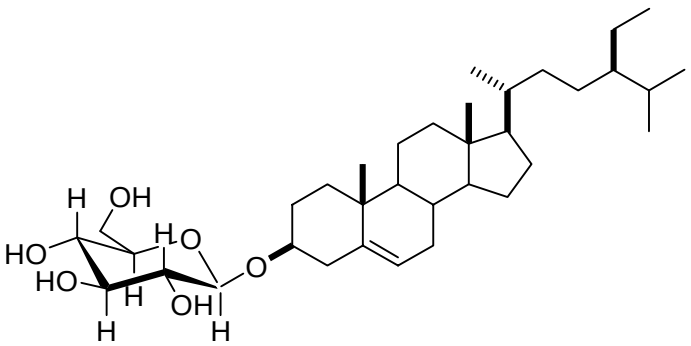
4.1.2.12 Identification of ME13

ME13 crystallised from acetone to give a colorless powder, mp 258-260 °C. It gave a positive response to the Liebermann-Burchard test for steroids. It also gave a blue-violet colour when heated with 10% H_2SO_4 in MeOH after TLC which served as a hint towards glycosylated compounds. The ESI mass spectrum showed peaks at *m/z* 599 $[M+Na]^+$, which suggested M to be 576. The EI mass spectrum also gave a peak at *m/z* 414 $[M_{aglycone}]^+$. These findings lead to a the molecular formula of $C_{35}H_{60}O_6$.

The ^1H NMR spectrum of **ME13** was very similar to that of **ME8** (**92**). The ^{13}C NMR also contained resonances of 35 carbon atoms which were assigned after examination of the DEPT and HMQC spectra as six methyl groups, 12 methylene groups, 14 methine groups and three quaternary carbon atoms. The only difference between the NMR spectra of **ME8** (**92**) and those of **ME13** was the presence of characteristic signals for the β-D-glucoside in **ME13** instead of signals of β-D-galactoside (see Table 4.4 for complete ^{13}C NMR and Experimental Section, page 111 for ^1H NMR data). These signals were deduced to be characteristic of β-D-glucoside on the basis of spectral analysis and by comparison with the reported data for sitosteryl β-D-glucoside (Sakakibara et al., 1983) and methyl β-D-glucoside (Seo et al., 1978).

The IR spectrum showed a broad band at $\tilde{\nu}$ 3398 cm^{-1} (OH). Additional peaks were observed at $\tilde{\nu}$ 2933, 2868, 1640, 1464, 1378, 1163, 1105, 1075 and 1024 cm^{-1}.

ME13 was finally identified as sitosteryl β-D-glucopyranoside (**25**) by direct comparison of its mp and spectroscopic data.



25

Table 4.4: ^{13}C-NMR data of steroidic compounds **24**, **25**, **26**, **91**, **92**.

	24	**25**	**26**	**91**	**92**
Atom no.	^{13}C-NMR ($CDCl_3$)	^{13}C-NMR (C_5D_5N)	^{13}C-NMR ($CDCl_3$)	^{13}C-NMR ($CDCl_3$)	^{13}C-NMR (DMSO-D_6)
1	37.22; CH_2	37.54; CH_2	37.14; CH_2	37.23; CH_2	36.73; CH_2
2	31.55; CH_2	30.31; CH_2	27.98; CH_2	31.63; CH_2	33.23; CH_2
3	70.61; CH	78.64; CH	71.07; CH	71.78; CH	76.78; CH
4	42.30; CH_2	40.01; CH_2	33.89; CH_2	42.27; CH_2	38.20; CH_2
5	140.71; C	140.98; C	40.26; CH	140.70; C	140.33; C
6	121.70; CH	121.94; CH	29.65; CH_2	121.70; CH	121.10; CH
7	31.60; CH_2	32.22; CH_2	117.43	31.89; CH_2	31.27; CH_2
8	31.80; CH	32.12; CH	139.57; C	31.87; CH	31.31; CH
9	50.10; CH	50.42; CH	49.45; CH	50.10; CH	49.50; CH
10	36.50; C	36.98; C	34.20; C	36.48; C	36.11; C
11	21.00; CH_2	21.33; CH_2	21.55; CH_2	21.20; CH_2	20.49; CH_2
12	39.70; CH_2	39.40; CH_2	39.46; CH_2	39.74; CH_2	39.13; CH_2
13	42.20; C	42.54; C	43.29; C	42.29; C	41.74; C
14	56.80; CH	56.89; CH	55.1; CH 3	56.74; CH	56.08; CH
15	24.30; CH_2	24.55; CH_2	23.07; CH_2	24.28; CH_2	23.76; CH_2
16	28.30; CH_2	28.57; CH_2	28.52; CH_2	28.23; CH_2	29.16; CH_2
17	55.90; CH	56.32; CH	55.90; CH	56.00; CH	55.32; CH
18	11.80; CH_3	12.02; CH_3	12.06; CH_3	11.84; CH_3	11.58; CH_3
19	19.40; CH_3	19.46; CH_3	13.05; CH_3	19.38; CH_3	18.82; CH_3
20	36.10; CH	36.43; CH	40.84; CH	40.48; CH	35.39; CH
21	18.60; CH_3	19.06; CH_3	21.10; CH_3	21.06; CH_3	18.51; CH_3
22	33.89; CH_2	34.28; CH_2	138.18;CH	138.30; CH	33.23; CH_2
23	26.03; CH_2	26.50; CH_2	129.44;CH	129.20; CH	25.30; CH_2
24	45.78; CH	46.12; CH	51.25; CH	51.22; CH	45.03; CH
25	29.11; CH	29.55; CH	31.88; CH	31.87; CH	28.57; CH
26	21.20; CH_3	19.27; CH_3	19.84; CH_3	21.00; CH_3	19.00; CH_3
27	19.00; CH_3	20.00; CH_3	19.04; CH_3	19.00; CH_3	19.61; CH_3
28	23.00; CH_2	23.46; CH_2	25.41; CH_2	26.00; CH_2	22.48; CH_2
29	12.00; CH_3	12.20; CH_3	12.26; CH_3	11.96; CH_3	11.68; CH_3
1’		102.63; CH			100.68; CH
2’		75.37; CH			73.35; CH
3’		78.48; CH			76.66; CH
4’		71.78; CH			69.97; CH
5’		78.19; CH			76.66; CH
6’		62.91			60.97

4.1.2.13 Identification of ME14

ME14 was isolated as a brown powder, mp 87-90 °C. Its molecular formula, $2(C_{15}H_{14}O_6)$, as established by ESI-FT-ICR mass spectrometry (*m/z* found: 603.14855; calcd. for $(C_{15}H_{14}O_6)_2$+Na: 603.14730) showed that it was a mixture of two compounds. As a matter of fact, on the ESI mass spectrum, two distinctive peaks were observed at *m/z* 313.1 $[M+Na]^+$ and 602.8 (base peak) $[2M+Na]^+$.

Its ^1H NMR spectrum was superposable to that of (+)-catechin (**97**) isolated from *Autranella congolensis*.

The ^{13}C NMR spectrum of **ME14** showed a series of 15 pairs of carbon signals which were sorted by DEPT and HMQC techniques (see Table 4.5).

ME14 was additionally identified as catechin by direct comparison with an authentic sample, both having the same R_f 0.32 on TLC in the mixture of CH_2Cl_2/MeOH 17:3.

A comparison of these chemical shifts with those reported for catechin (Nay et al., 2001 and 2002) (see Table 4.5) showed that **ME14** was a mixture of (+)-catechin (**97**) and (-)-epicatechin (**98**).

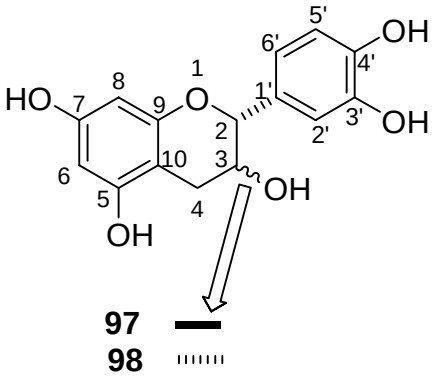


Table 4.5: NMR data of compounds **97** and (**97**+**98**)

	97[a]	**97**	**97**+**98**	
Atom no	^{13}C-NMR (CD_3OD)	^{13}C-NMR (CD_3OD)	^{13}C-NMR (DMSO-D_6)	^{1}H-NMR (DMSO-D_6)
2	82.7	79.90; CH	80.92 + 77.98	4.47 + 4.77
3	68.8	67.51; CH	66.22 + 64.83	3.82 + 4.02
4	28.4	29.30; CH_2	28.12 + 27.78	2.48 + (2.68; 2.36)
5	157.7	158.04; C	156.45 + 156.36	
6	95.6	96.40; CH	94.99	5.89
7	157.5	157.69; C	156.15 + 156.09	
8	96.4	95.91; CH	93.97	5.69
9	156.8	157.40; C	155.68 + 155.26	
10	100.9	100.09; C	98.93 + 98.37	
1’	132.2	132.31; C	130.47 + 130.43	
2’	115.3	115.34; CH	114.66 + 114.42	6.72
3’	146.2	145.92; C	144.46 + 144.42	
4’	146.2	145.97; C	144.82	
5’	116.2	115.91; CH	114.96 + 114.80	6.67 + 6.89
6’	120.1	119.42; CH	118.26 + 117.81	6.59 + 6.67

[a] ^{13}C-NMR (CD_3OD) from literature (Nay et al., 2001)

4.2 Isolation and Characterisation of compounds from *Autranella congolensis*

The crude extract of *Autranella congolensis* was fournished by a collaborator, Dr. Nkeng-Efouet A. P. from the University of Dschang in Cameroon.

4.2.1 Extraction

Plant material was collected in the West Province of Cameroon, cut into pieces, air-dried and pulverized. The resulting powder has been extracted and chromatographed following the scheme below:

4.2.2 Identification of compounds

4.2.2.1 Identification of Hex_F2

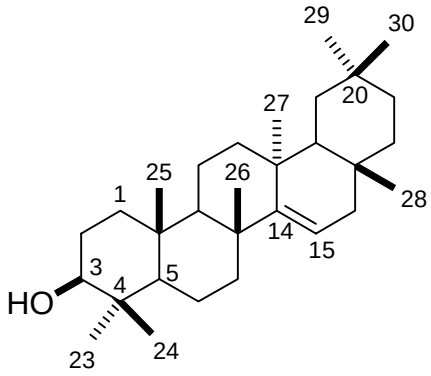
Hex_F2 crystallised from acetone to give a colorless powder (170 mg), mp 276-278 °C. It gave a positive response to the Liebermann - Burchard test for terpenoids. Its EI mass spectrum indicated a molecular ion at *m/z* 426 [M^+] confirming to the molecular formula $C_{30}H_{50}O$, as established by HRMS (EI) (*m/z* found: 426.38609 [M^+]; calcd.: 426.38617 [M^+]), which corresponded to six double bonds equivalent.

The ^{1}H NMR spectrum of **Hex_F2** contained resonances corresponding to eight methyl groups in the region δ 0.90 to δ 1.13, all as singlets. The ethylenic proton (H-15) resonated as a double doublet at δ 5.63 (dd, *J* = 8.2, 3.1 Hz) and the signal of one exchangable hydroxy group was observed as a doublet at δ 5.80 (*J* = 5.7 Hz). According to the fact that naturally occurring polyoxygenated triterpenoids in general contain an oxygen function at position 3 (Gunasekera and Sultanbawa, 1977; Basu and Rastogi, 1967 and Agarwal and Rastogi, 1974) the single hydroxy group was placed at that position. The observation of the base peak at *m/z* 204 (100) in the mass spectrum and the characteristic double doublet signal of the single olefinic proton in the ^{1}H NMR spectrum suggested the compound to be a Δ^{14}-pentacyclic triterpenoid (Ageta and Arai, 1983). The broad band decoupled ^{13}C NMR spectrum of **Hex_F2** showed 30 carbon signals which were assigned by DEPT and HMQC techniques as eight methyl groups, 10 methylene groups, five methine groups among them one bearing an oxygen (δ 78.2, C-3) together with seven quaternary carbons (see Table 4.6 for complete ^{13}C NMR data). The resonances of the olefinic carbons at δ 158.4 and 117.1 were also found to be characteristic of Δ^{14}-pentacyclic triterpenes (Sakurai et al., 1987).

A closer look on the mass spectra revealed the presence of some major fragments that confirmed the localisation of the double bond at C-14. In particular the peak at *m/z* 302 is due to the retro-Diels Alder reaction as has been observed in a similar fashion with the Δ^7-unsaturated derivatives, except that ring D rather than ring C is being opened (see arrows on Figure 4.7). Here the charge remains again with the diene portion, which now comprises rings A, B, C. This mechanism was described by Budzikiewicz et al., 1963, as well as the occurrence of the base peak at *m/z* 204 (100). The authors explained the occurrence of this base peak by assuming that in the molecular ion the missing electron is preferentially removed from the carbon-carbon double bond, followed by the migration of

the C-13 methyl group to C-14; fission of the 11-12 and 8-14 bonds now gives the stable diene (*m/z* 204) (Figure 4.8).

Finally, **Hex_F2** was identified through a comparison (see Table 4.6) of its spectroscopic data and mp (Lit. 273-274 °C, Agarwal et al., 1963; 278-279 °C, Sakurai et al., 1987) to the literature data (Mahato and Kundu., 1994; Agarwal et al., 1963; Sakurai et al., 1987) as taraxerol (**99**).



99

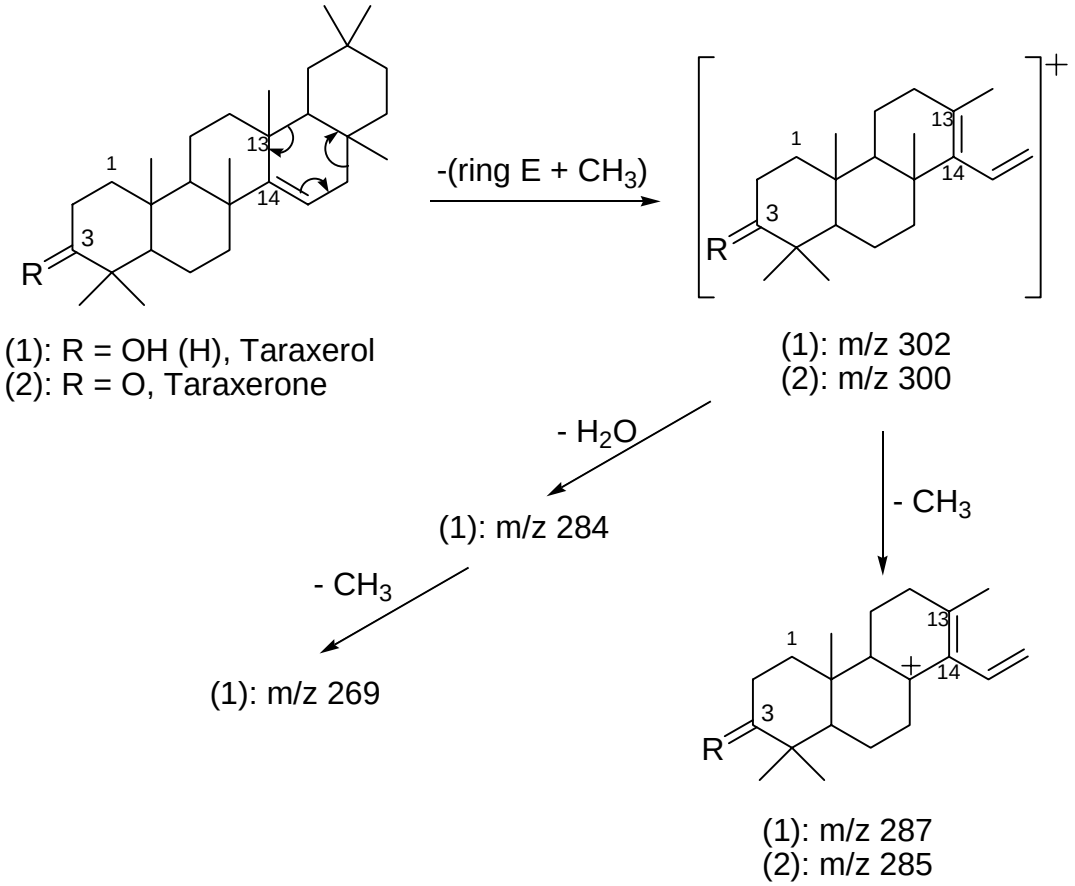


Figure 4.7: Fragmentation mechanism of **Hex_F2** (**99**) and **AC_Hex.2.A** (**100**)

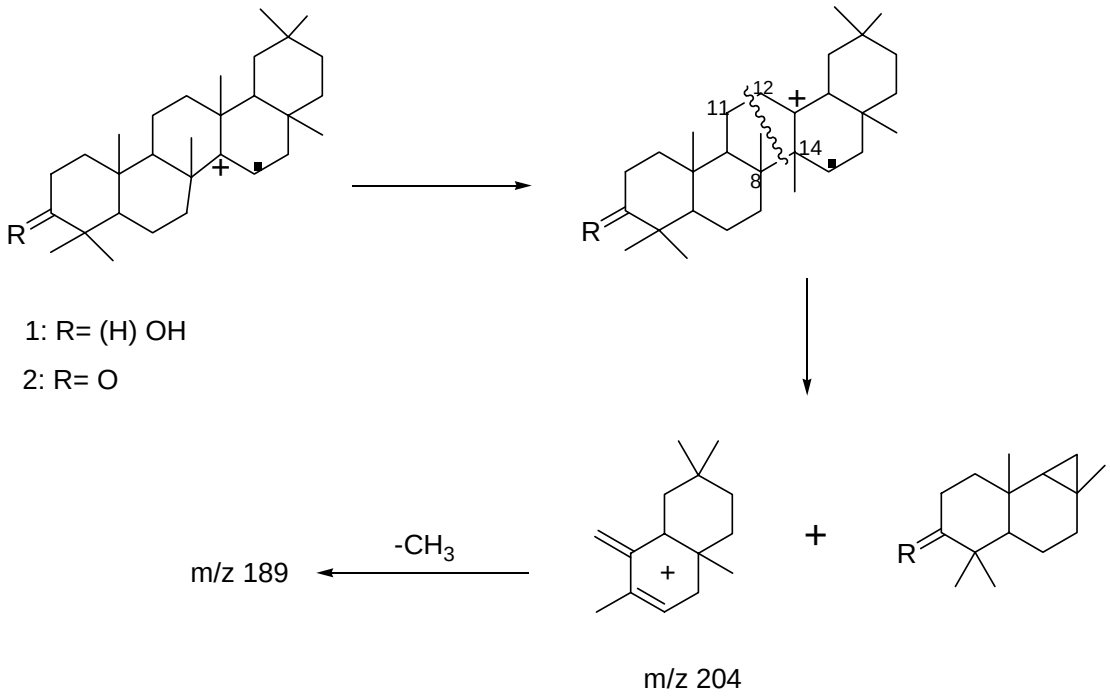


Figure 4.8: Occurrence of the base peak in Δ^{14}-pentacyclic triterpenes

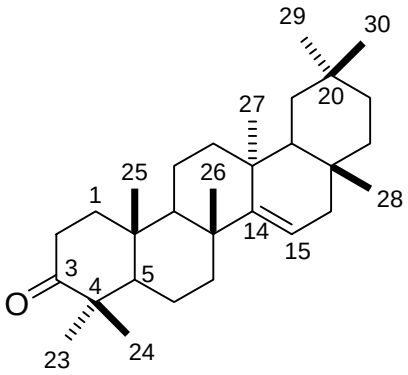
4.2.2.2 Identification of AC_Hex2.A

AC_Hex2.A crystallised from acetone to give a colorless powder (60 mg), mp 240-242 °C. It gave a positive response to the Liebermann - Burchard test for terpenoids. Its ESI mass spectrum indicated a molecular ion at *m/z* 425 [M+H$^+$] and *m/z* 849 [2M+ H$^+$]. Its molecular formula $C_{30}H_{48}O$, as established by ESI-FT-ICR mass spectrometry (*m/z* found: 425.37747; calcd. for $C_{30}H_{49}O$: 425.37779 and *m/z* 447.35936; calcd. for $C_{30}H_{48}O$ + Na: 447.35974), corresponded to seven double bond equivalents.

The NMR spectra were so similar to those of taraxerol described above that the carbon skeleton could be deduced to be identical to the taraxerol one. The characteristic double doublets signal of an olefinic proton [δ 5.53 (dd, *J* = 8.2, 3.1 Hz, H-15)] and the eight singlet signals of methyl group protons in the ^{1}H NMR spectrum also strongly suggested **AC_Hex2.A** to be a derivative of taraxer-14-ene. The only difference in its ^{13}C NMR spectrum was the presence of a carbonyl group for a ketone (δ 217.6) and the absence of a signal for a methine group bearing an oxygen substituent (see Table 4.6 for complete ^{13}C NMR data). This ketone was deduced to be derived from taraxerol, and therefore the carbonyl group was localised at C-3.

The IR spectrum showed peaks at $\tilde{\nu}$ 3047 cm^{-1} (olefinic CH), 2939, 2863 (aliphatic CH), a strong and sharp peak at $\tilde{\nu}$ 1708 (carbonyl). The peak at $\tilde{\nu}$ 1376 cm^{-1} was indicative for two geminal methyl groups.

Finally a comparison (see Table 4.6) of its mp (Lit. 240-243 °C, Sakurai et al., 1987) and NMR data to those obtained from the literature for taraxerone (Sakurai et al., 1987) confirmed that AC_Hex2.A was taraxerone (**100**).



100

4.2.2.3 Identification of Hex_F1

This compound crystallised from a mixture of PE-CH_2Cl_2 was obtained as a colorless powder, mp 176-178 °C, soluble in chloroform. It gave a positive response to the Liebermann - Burchard test for terpenoids. Its ESI mass spectrum contained peaks at *m/z* 805, 777 and 749, while the EI mass spectrum indicated particular peaks at *m/z* 804, 776 and 748, suggesting that **Hex_F1** was a mixture of three compounds which differ one from another by a mass difference of 28 corresponding to two methylene groups.

The APCI-FT-ICR mass spectrometry confirmed that **Hex_F1** was composed of three compounds with the molecular formulas $C_{56}H_{100}O_2$, $C_{54}H_{96}O_2$ and $C_{52}H_{92}O_2$, respectively for *m/z* 805.78011; calcd. for $C_{56}H_{101}O_2$: 805.77961, *m/z* 777.74865; calcd. for $C_{54}H_{97}O_2$: 777.74831 and *m/z* 749.71776; calcd. for $C_{52}H_{93}O_2$: 749.71701, each corresponding to seven double bond equivalents.

Its ^1H NMR and ^{13}C NMR spectra were very similar to those of Hex_F2. In effect, the ^1H NMR spectrum displayed a signal of one proton at δ 4.46 (H-3, dd, J = 10.4, 5.3 Hz) indicating the struture fragment C(sp^3)H-OCO-, together with one signal at δ 1.25-1.26 corresponding to a series of methylene groups. The triplet at δ 2.28 (J = 7.5 Hz) was

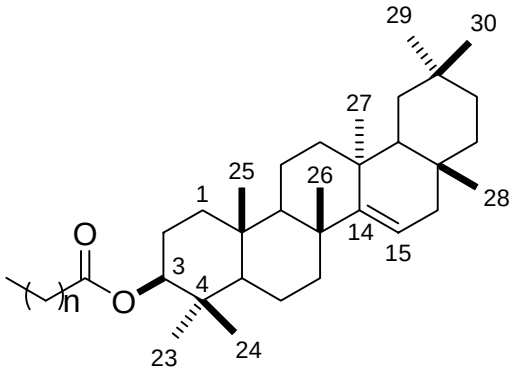
assigned to a methylene group located at the α-position of the carbonyl group whereas the one at δ 0.88 (J = 5.9 Hz) was due to the terminal methyl of an aliphatic chain (Öksüz and Topcu, 1987). All those signals indicated the presence of a long aliphatic chain of a fatty acid esterified in postion 3 of taraxerol. This conclusion derived from what Cambie et al. described in the literature for the esterified β-amyrine esters (Cambie et al., 1977). This suggestion was confirmed after examination of the broad band decoupled ^{13}C and DEPT spectra which showed the following signals:

- δ 29.17-29.82, long aliphatic chain assigned to methylene groups.
- δ 14.1, terminal methyl group of a long aliphatic carbon chain (Chavez et al., 1996).
- δ 34.9, triplet assigned to the methylene group located at the α-position of the carbonyl group (Wansi, 2000).

In addition the C-3 here resonated at lower field (δ 80.6) than that of taraxerol (δ 78.2). The complete ^{13}C NMR data are reported in Table 4.6.

Alkaline hydrolysis of Hex_F1 yielded a mixture of fatty acids and the triterpene which was identified as taraxerol by spectral data as well as by direct comparison with the authentic sample (TLC, R_f 0.34, CH_2Cl_2/PE 1:1).

From all those physical and spectroscopic data, Hex_F1 was identified as a mixture of 3β-docosanoyltaraxer-14-ene (n = 20), 3β-tetracosanoyltaraxer-14-ene (n = 22), 3β-hexacosanoyltaraxer-14-ene (n = 24) (**101**).



101 n = 20, 22, 24

Table 4.6: ^{13}C NMR data of compounds (**99**), (**100**) and (**101**)

	99	**99[a]**	**100**	**100[a]**	**101**
Atom no	^{13}C NMR (C_5D_5N)	^{13}C NMR ($CDCl_3$)	^{13}C NMR ($CDCl_3$)	^{13}C NMR ($CDCl_3$)	^{13}C NMR ($CDCl_3$)
1	38.22; CH_2	38.1	38.34; CH_2	38.4	37.35; CH_2
2	28.02; CH_2	27.3	34.14; CH_2	34.1	25.17; CH_2
3	78.15; CH	79.2	217.60; CH	217.3	80.63; CH
4	39.41; C	39.1	47.58; C	47.6	37.71; C
5	55.96; CH	55.7	55.76; CH	55.8	55.61; CH
6	19.20; CH_2	19.0	19.95; CH_2	20.0	18.68; CH_2
7	35.32; CH_2	35.3	35.08; CH_2	35.2	35.09; CH_2
8	39.29; C	38.9	38.88; C	38.9	38.97; C
9	49.08; CH	48.9	48.68; CH	48.7	48.72;CH
10	37.78; C	37.9	37.52; C	37.6	37.87; C
11	17.84; CH_2	17.7	17.44; CH_2	17.5	17.51; CH_2
12	36.89; CH_2	36.9	35.77; CH_2	36.7	37.68; CH_2
13	37.92; C	37.9	37.68; C	37.7	37.53; C
14	158.41; C	158.1	157.58; C	157.6	157.97; C
15	117.11; CH	117.0	117.18; CH	117.2	116.92;CH
16	36.04; CH_2	35.9	36.65; CH_2	35.8	36.64; CH_2
17	38.27; C	38.1	37.73; C	37.7	35.78; C
18	49.52; CH	49.4	48.75; CH	48.8	49.16; CH
19	41.65; CH_2	41.4	40.61; CH_2	40.7	41.19; CH_2
20	28.98; C	29.0	28.79; C	28.8	28.79; C
21	33.99; CH_2	33.9	33.55; CH_2	33.6	33.66; CH_2
22	33.38; CH_2	33.2	33.06; CH_2	33.1	33.08; CH_2
23	28.67; CH_3	28.1	26.08; CH_3	26.2	27.99; CH_3
24	16.44; CH_3	15.6	21.48; CH_3	21.5	16.67; CH_3
25	15.70; CH_3	15.6	14.80; CH_3	14.8	15.42; CH_3
26	30.04; CH_3	30.1	29.84; CH_3	29.9	29.92; CH_3
27	26.18; CH_3	26.0	25.57; CH_3	25.6	25.92; CH_3
28	30.04; CH_3	30.1	29.92; CH_3	29.9	29.92; CH_3
29	33.44; CH_3	33.5	33.35; CH_3	33.4	33.34; CH_3
30	21.52; CH_3	21.5	21.34; CH_3	21.4	21.28; CH_3
$\underline{C}OO$-					173.69
$\underline{C}H_2COO$-					34.86
$(CH_2)_n$					29.82-29.17
CH_3					14.14

[a] ^{13}C-NMR ($CDCl_3$) from literature (Sakurai et al., 1987)

4.2.2.4 Identification of AC_F32

This compound crystallised in the mixture of PE-EtOAc as a brown powder, mp 237-239°C, soluble in MeOH. Its molecular formula $C_{15}H_{14}O_6$, was established by HRMS (EI) (*m/z* calcld: 290.07815, found: 290.07904 [M^+]) and corresponded to nine double bonds equivalents.

Its ^1H NMR spectrum in CD_3OD exhibited signals of five aromatic protons where the coupling pattern between those appearing at δ 6.96 (1H, d, *J* = 1.9 Hz, H-2'), δ 6.79 (1H, dd, *J* = 8.2, 1.9 Hz, H-6') and δ 6.75 (1H, d, *J* = 8.2 Hz, H-5') indicated a 1,3,4-trisubstituted aromatic ring, whereas those appearing at δ 5.94 (1H, d, *J* = 2.0 Hz, H-6) and δ 5.91 (1H, d, *J* = 2.0 Hz, H-8) were indicative of a 1,2,3,5-tetrasubstituted aromatic ring. Further signals were observed at δ 4.82 (1H, d, *J* = 6.5 Hz, H-2), δ 4.17 (1H, m, H-3), δ 2.85 (1H, dd, *J* = 16.8, 4.6 Hz) and δ 2.73 (1H, dd, *J* = 16.8, 2.7 Hz). The large coupling constant (*J* = 6.5 Hz) of H-2 with H-3 indicated that this unit had the 2,3 *trans* configuration.

The broad band decoupled ^{13}C NMR spectrum of AC_F32 shows 15 carbon signals which were assigned by DEPT and HMQC techniques as seven methine groups, seven quaternary carbons among them five bearing an oxygen and one methylene groups (δ 29.3, C-4). A comparison of all these spectral data with those of some flavonoids (Rensburg et al., 2000; Steynberg et al., 1995) led to the conclusion that AC_F32 should have the basic skeleton of flavonoids. These NMR data showed similarities to those of (+)-catechin (Nayet al., 2001) after comparison (see Table 4.5).

Its IR spectrum showed important peaks at $\tilde{\nu}$ 3455, 3164, 2931, 1625 and 1520. From all these informations above and, this compound was finally identified as (+)-catechin (**97**).

4.2.2.5 Identification of Hex_C.1

Hex_C.1 crystallised from a mixture of PE-CH_2Cl_2 as a white powder, mp 156-158 °C, soluble in chloroform. It gave a positive response to the Liebermann - Burchard test for steroids. Its ESI mass spectrum indicated peaks at *m/z* 413 $[M+H]^+$, whereas the EI mass spectrum indicated the molecular ion peak at *m/z* 412 $[M]^+$, leading to the molecular formula $C_{29}H_{48}O$, as established by HRMS (EI) (*m/z* calcd.: 412.37047 [M^+]), found: 412.37052 [M^+]); this corresponded to six double bond equivalents.

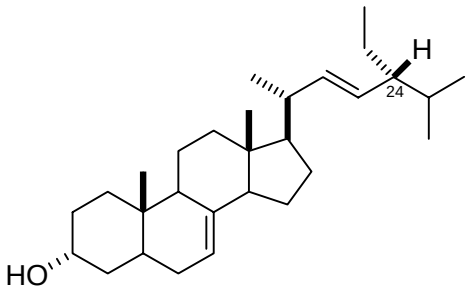
Its ^{1}H NMR spectrum exhibited signals of olefinic protons at δ 5.03 (1H, ddd, *J* = 16.0, 7.0, 7.0 Hz, H-23), δ 5.15 (1H, dd, *J* = 16.0, 7.0 Hz, H-22), and δ 5.15 (1H, m, H-7), a methine proton at δ 3.6 (1H, m, H-3), and six methyl groups of wich two were singlets at δ 0.55 and δ 0.81, one triplet at δ 0.84 (3H, t, *J* = 6.5 Hz), and the three other as doublets at δ 0.86, δ 0.93 and δ 1.03, having a coupling constant in the range 6.5-7.6 Hz.

Its broad band decoupled ^{13}C NMR spectrum showed 29 carbon signals which were assigned by DEPT and HMQC techniques. The particular signals at δ 129.4 and δ 138.2 were characteristic of a Δ^{22}-sterol (Rubinstein et al., 1976), whereas those at δ 117.4 and δ 139.6 where characteritic of a Δ^{7}-sterol (Jahan et al., 1995). The methine proton showed a cross peak in the HMQC with the carbon signal at δ 71.1, which was assigned to C-3 on biogenetic grounds of steroids. The complete ^{13}C NMR data are reported on Table 4.4.

The EIMS showed further fragments at *m/z* 273 $[M\text{-side-chain}]^{+}$, *m/z* 271 $[M\text{-(side-chain)-2H}]^{+}$, *m/z* 255 $[M\text{-(side-chain)-}H_2O]^{+}$, *m/z* 253 $[M\text{-(side-chain)-}H_2O\text{-2H}]^{+}$, 95, 83 and 81 (see Figure 4.9). The last fragments *m/z* 95, 83 and 81 were also reported to be characteristic of Δ^{22}-sterols (Laine and Elbein, 1971).

The *R* configuration at C-24 was established by the method of Itoh et al.(1981), based on the comparison of the ^{1}H and ^{13}C- NMR spectra with steroids related to α-spinasterol and chondrillasterol.

Finally, **Hex_C.1** was identified as (24*R*)-stigmast-7,22(*E*)-dien-3α-ol (**26**), reported previously by Itoh et al. (1981).



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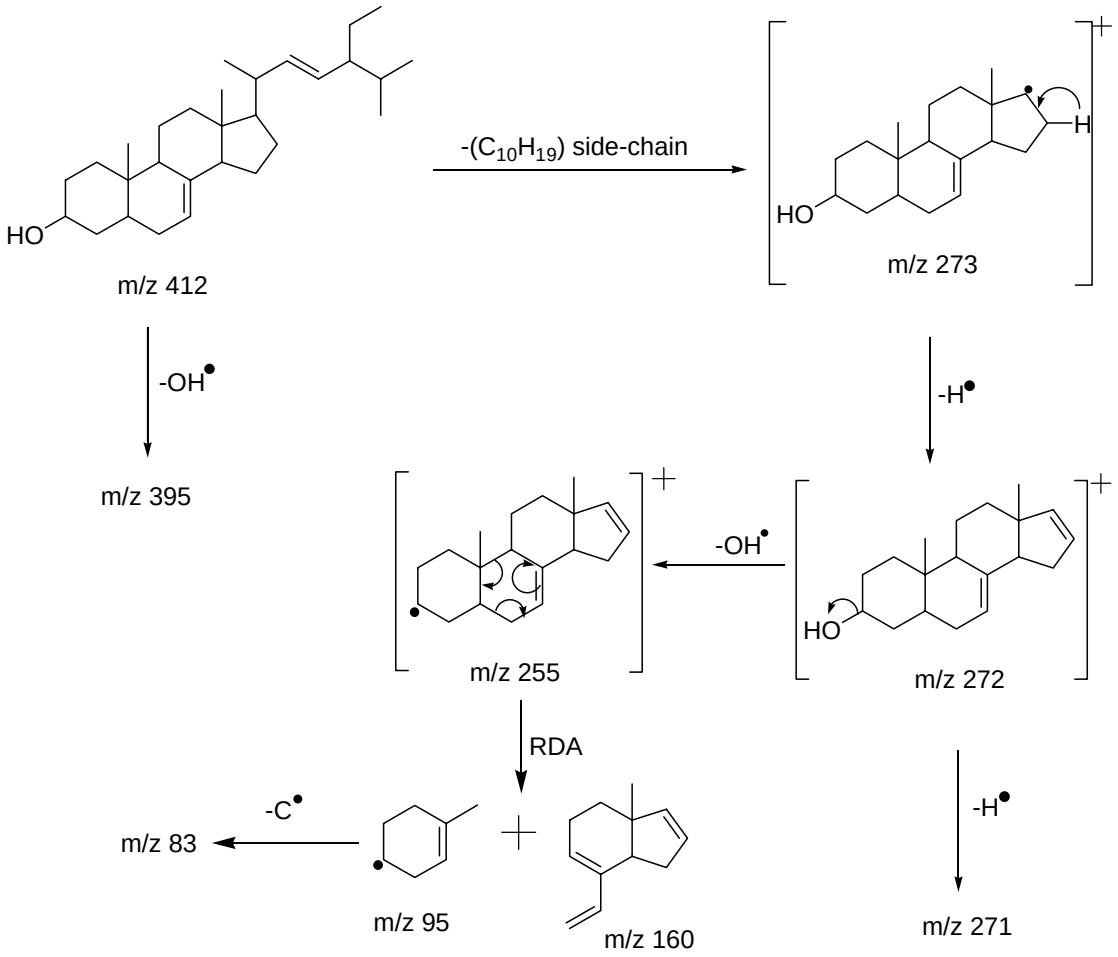


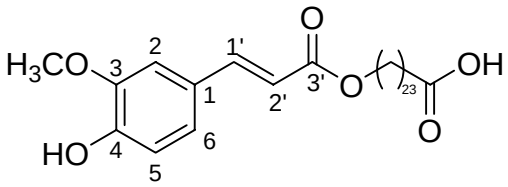
Figure 4.9: Fragmentation mechanism of **Hex_C.1** (**26**)

4.2.2.6 Identification of AC_F7.2

AC_F7.2 crystallised as a colorless powder in chloroform, mp 82-85 °C. Its ^{1}H NMR spectrum showed similarities to that of **ME9** (**93**) isolated from *Maesopsis eminii*. However, it was composed of two moieties instead of three as **ME9**. One was trans-4-hydroxy-3-methoxycinnamate, with aromatic protons at δ 6.91 (d, *J* = 8.2 Hz), δ 7.03 (d, *J* = 1.9 Hz) and δ 7.07 (dd, *J* = 8.2, 1.9 Hz); a methoxy group at δ 3.91 and one ethylene trans: δ 6.29 (d, *J* = 15.7 Hz) and δ 7.6 (d, *J* = 15.7 Hz). The second moiety showed two distinctive methylene groups connected to an oxycarbonyl (δ 4.18, t, *J* = 6.9 Hz) and to carbonyloxy (δ 2.34, t, *J* = 7.5 Hz); two methylene groups connected to those described above (δ 1.7, t and δ 1.6, m); one further methylene group (δ 1.37, m), and the rest of the methylene groups as a long aliphatic chain (δ 1.24-1.26, br s).

The ESI mass spectrum (positive mode) of **AC_F7.2** gave a pseudomolecular peak at *m/z* 561 $[M+H]^+$. The ESI mass spectrum (negative mode) contained a peak at *m/z* 559 $[M-H]^-$, corresponding to the molecular formula $C_{34}H_{56}O_6$, corresponding to seven double bond equivalents; and *m/z* 531. Its EI MS also showed the base peak at *m/z* 177, and other signals at *m/z* 560 $[M]^+$, 514, 486, 396, 368 and 194. These fragments can be explained by the same fragmentation mechanism as for **ME9** (**93**) (Figure 4.5).

The ^{13}C NMR spectrum of **AC_F7.2** showed a series of carbon signals which were identified by DEPT and HMQC techniques as five quaternary carbons including two carbons (δ 178.80 and δ 167.45) of two carbonyl groups, five methine groups, one methoxy group (δ 55.93), six methylene groups of which one was oxygenated, and additional methylene groups corresponding to a long aliphatic chain (δ 29.70 – 29.07). A comparison of these data with those reported for a mixture of $C_{22\text{-}28}$ ω-hydroxy acid esters of cis and trans-ferulic acid showed that **AC_F7.2** was 24-feruloyltetracosanoic acid (**102**) (Kawanishi and Higashimoto, 1987; Hiramoto and Wanatabe, 1939).



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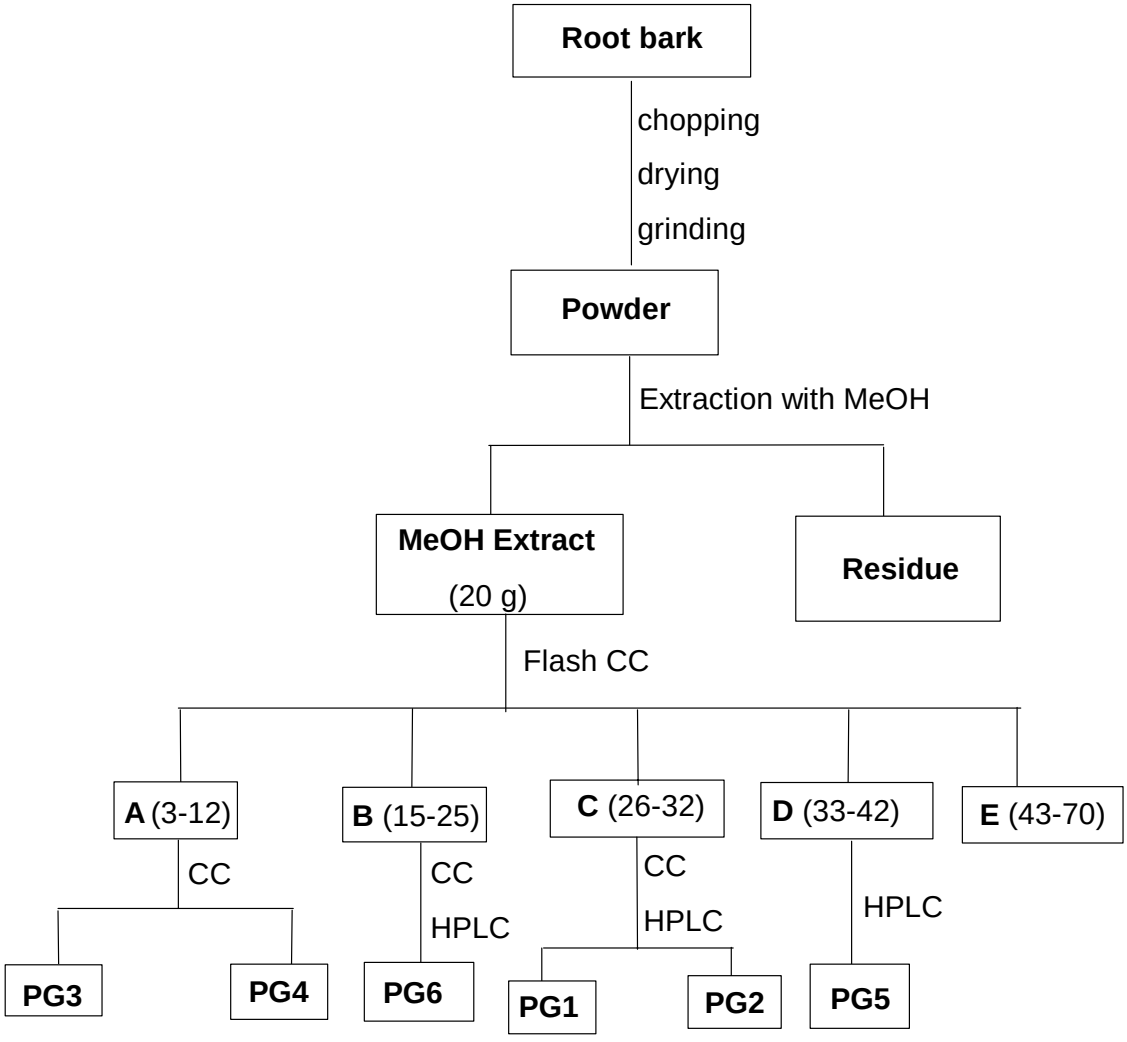
4.3 Isolation and Characterisation of compounds from *Pentadesma grandifolia*

The crude extract of *Pentadesma grandifolia* was provided by a collaborator, Mr. Hilaire V. Kemami Wangun, from the Hans-Knöll-Institut for Natural Product Research in Jena, Germany.

4.3.1 Extraction

Plant material was collected in the West Province of Cameroon, cut into pieces, air-dried and pulverized. The resulting powder has been extracted and further purified chromatographically following the scheme below:

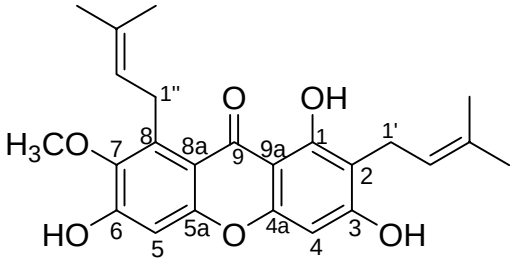


4.3.2 Identification of compounds

4.3.2.1 Identification of PG1

PG1 was obtained as a yellow gum (200 mg), and reacted positively with alcoholic ferric chloride, indicating its phenolic nature. It exhibited a molecular ion [M^+] at *m/z* 410.17240, leading to the molecular formula $C_{24}H_{26}O_6$ (calcd. for 410.17294), whish corresponds to 12 double bond equivalents. The ^{1}H NMR spectrum of **PG1** displayed signals that suggested a polyhydroxylated polyprenylated xanthone structure (Likhitwitayawuid et al., 1997; Pattalung et al., 1994; Lee and Chan, 1977; Sakai et al., 1993). It showed, in the downfield region, two aromatic proton resonances at δ 6.18 (s, H-4) and δ 6.63 (s, H-5), as well as a signal for three OH at δ 4.87 (br s). Two prenyl groups were present in the structure of **PG1**, as evident from the following resonances: (a) two olefinic protons at δ 5.21 (m, H-2' and H-2''); two pairs of methylene protons at δ 3.24 (d, J = 7.5 Hz, H-1') and δ 4.02 (d, J = 6.3 Hz, H-1''); and four methyl groups at δ 1.64 (s, H_3-4'), 1.65 (s, H_3-4''), 1.76 (s, H_3-5'), 1.80 (br s, H_3-5''). The signal of a methoxy group was observed at δ 3.73 (s, 7-OMe). The nature of allylic and homoallylic coupling systems within each isoprene moiety was explicitly demonstrated in the HH-COSY spectrum. In fact, the ^{1}H NMR spectrum of PG1 possessed very close resemblance to that of 1,3,6-trihydroxy-7-methoxy-2,5,8-triprenylxanthone (Likhitwitayawuid et al., 1997), except for the absence of a third prenyl moiety located at C-5 in **PG1**. The presence of six oxygenated aromatic carbon signals on the ^{13}C NMR spectrum at δ 161.58 (C-1), 163.57 (C-3), 157.79 (C-6), 156.67 (C-5a), 156.16 (C-4a) and 144.73 (C-7) also supported the presence of a xanthone skeleton in **PG1**, which has been found to occur in some compounds in other genera in the family Guttiferae including *Pentadesma* (Gunasekera et al., 1977a, Nkengfack et al. 2002, Monache et al., 1983). The complete ^{13}C NMR data of PG1 are reported in Table 4.7. A HMBC NMR experiment was employed to determine the position of these two side chains in **PG1**. One of the prenyl groups was attached to C-2 based on the HMBC correlations of H-1'/C-2 (two bonds), H-1'/C-1 (three bonds), H-1'/C-3 (three bonds). The second prenyl group was found to be located at C-8 by the HMBC correlations of H-1''/C-8 (two bonds), H-1''/C-8a (three bonds) and H-1''/C-7 (three bonds). The HMBC correlation from C-7 to the methoxy protons also indicated the locations of the prenyl group at C-8, and the

methoxy group at C-7. Finally, **PG1** was identified as 1,3,6-trihydroxy-7-methoxy-2,8-diprenylxanthone, also known as α-mangostin (**103**) (Matsumoto et al., 2003).



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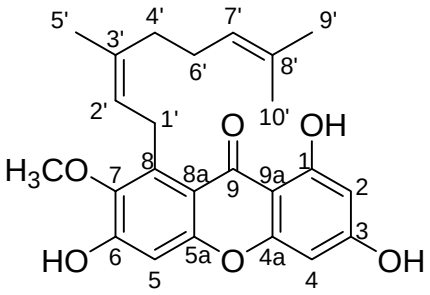
4.3.2.2 Identification of PG2

PG2 was isolated as a yellowish powder (130 mg), mp 205-207 °C. It was deduced to have an elemental formula of $C_{24}H_{26}O_6$, as established by HRMS (EI) which showed a molecular ion peak at *m/z* 410.17366 (calcd. 410.17294), and corresponds to 12 double bond equivalents. It also reacted positively with alcoholic ferric chloride, indicating its phenolic nature. Its ^{1}H and ^{13}C NMR spectra also exhibited signals which were suggestive of a polyhydroxylated xanthone structure (see Table 4.7), thus it was deduced to be an isomer of PG1. In effect, signals at δ_H 4.02/δ_C 27.03 (C-1'), 5.2/125.27 (C-2'), 1.96/40.82 (C-4'), 1.80/16.66 (C-5'), 2.04/27.61 (C-6'), 5.04/127.51 (C-7'), 1.54/25.80 (C-9'), 1.51/17.73 (C-10'), δ_C 135.45 (C-3') and 131.99 (C-8') were found to be due to the presence of a geranyl group, by comparison of the NMR data with the literature values (Sia et al., 1995; Seo et al., 2002; Lee and Ng, 1982); a signal of a methoxy group appeared at δ_H 3.76/δ_C 61.41. In the ^{1}H NMR spectrum of PG2, the signal of H-1' of this geranyl group appears further downfield δ_H (4.02) than the usual values of this functionality (Somanathan and Sultanbawa, 1972; Jackson et al., 1967). This can be explained from the fact that H-1' is in a region deshielded by the carbonyl group (Seo et al., 2002) which is consistent with the assigned position (C-8) of the geranyl group identified by HMBC correlations of H-1'/C-8, H-1'/C-8a and H-1'/C-7. Thus, **PG2** was identified to 1,3,6-trihydroxy-7-methoxy-8-geranylxanthone, named rubraxanthone (**104**) (Ampofo and Waterman, 1986).



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4.3.2.3 Identification of PG3

PG3 was a white powder (150 mg), mp 213-215 °C. It gives a positive response to the Liebermann - Burchard test for terpenoids. Its molecular formula $C_{30}H_{50}O$ was established by HRMS (EI) which exhibited the peak of the molecular ion at *m/z* 426.38650 [M^+], (calcd. 426.38617), and corresponds to six double bond equivalents. Other characteristic peaks were found at *m/z* 408 [$M-H_2O]^+$, 218, 189 on the EI-MS.

IR absorptions at 3318 and 1638 cm^{-1} indicated the presence of a hydroxyl group and a terminal double bond, while the band at 1378 cm^{-1} was indicative for two geminal methyl groups.

In the ^1H NMR spectrum the presence of the following moieties were deduced: a terminal methylene group [δ 4.56 (1 H, d, *J* = 2.0 Hz); δ 4.68 (1 H, d, *J* = 2.0 Hz)], one methyl group at δ 1.68 (3 H, s), (CH_3-C=C), and six other methyl groups in the region δ 1.03 - 0.76.

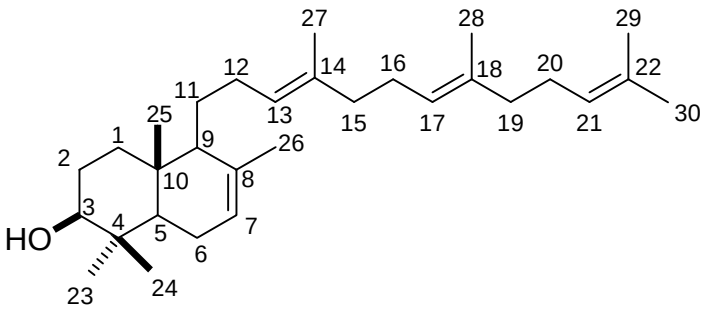
The broad band decoupled ^{13}C NMR spectrum of **PG3** shows 30 carbon signals which were assigned by DEPT and HMQC techniques as seven methyl groups, 11 methylene groups, six methine groups among them one bearing an oxygen (δ 78.97, C-3) and seven quaternary carbons. The carbon signals at δ 109.32 and 150.96 ppm were found to be characteristic of a $\Delta^{20(29)}$-pentacyclic triterpene (Mahato and Kundu, 1994; Reynolds et al., 1986).

The identity of **PG3** as lupeol (lup-20(29)-en-3β-ol) (**23**, page 13) was finally established by direct comparison of the mp (Lit. 215-216 °C, Reynolds et al., 1986), IR, EIMS, ^1H NMR and ^{13}C NMR data with those reported in the literature (Reynolds et al., 1986).

4.3.2.4 Identification of PG4

This compound was obtained as a yellow oil (1 g). Its molecular formula $C_{30}H_{50}O$ was established by ESI-FT-ICR mass spectrometry (*m/z* 427.39315; calcd. for $C_{30}H_{51}O$: 427.39344), corresponded to six double bond equivalents. Its IR spectrum showed a characteristic hydroxyl absorption band at 3376 cm^{-1}. It gave a positive response to the Liebermann - Burchard test for terpenoids. Its ^{1}H and ^{13}C NMR spectra contained signals of four trisubstituted double bonds [5.38 (1H, s, br), 5.12 (3H, m), H-7, H-13, H-17 and H-21; 121.91, 124.64, 124.40, 124.20 (each d, C-7, C-13, C-17 and C-21) and 135.40, 135.09, 134.94, 131.22 (each s, C-8, C-14, C-18 and C-22)], a secondary alcohol [3.22 (1H, dd, J = 4.6 and 11.1 Hz, H-3); 79.16 (d, C-3)], five vinyl methyl groups [1.71 (3H, br s, H-26), 1.68 (3H, br s, H-30), 1.61 (6H, br s, H-28 and H-29), 1.59 (3H, s, H-27); 25.71 (q, C-30), 22.01 (q, C-26), 17.69 (q, C-29), 16.17 (q, C-27), 16.01 (q, C-28)] and three quaternary methyl groups [0.96, 0.84, 0.74 (each 3H, br s, H-23, H-24 and H-25); 27.89, 15.08 and 13,50 (each q, C-23, C-24, C-25)]. In addition, signals of nine methylene groups, two methine groups and two quaternary carbons were displayed in these spectra. Therefore, the molecule is a bicyclic triterpenoid. Bicyclic triterpenoids are quite rare and comparison of the ^{1}H and ^{13}C NMR spectra of **PG4** with those of (13*E*,17*E*)-polypoda-8(26),13,17,21-tetraen-3β-ol and (13*E*,17*E*)-polypoda-7,13,17,21-tetraen-3β-ol both previously isolated from *Cratoxylum cochinchinense* (Bennett et al, 1993; Nguyen and Harrison, 1998) and γ-polypoda-7,13,17,21-tetraene isolated from *Polystichum ferns* (Shiojima et al., 1983) clearly indicated that **PG4** was (13*E*,17*E*)-polypoda-7,13,17,21-tetraen-3β-ol (**105**).

The MS fragmentations (Figure 4.10) were in good agreement with the presence of a farnesyl chain (Nguyen and Harrison, 1998).



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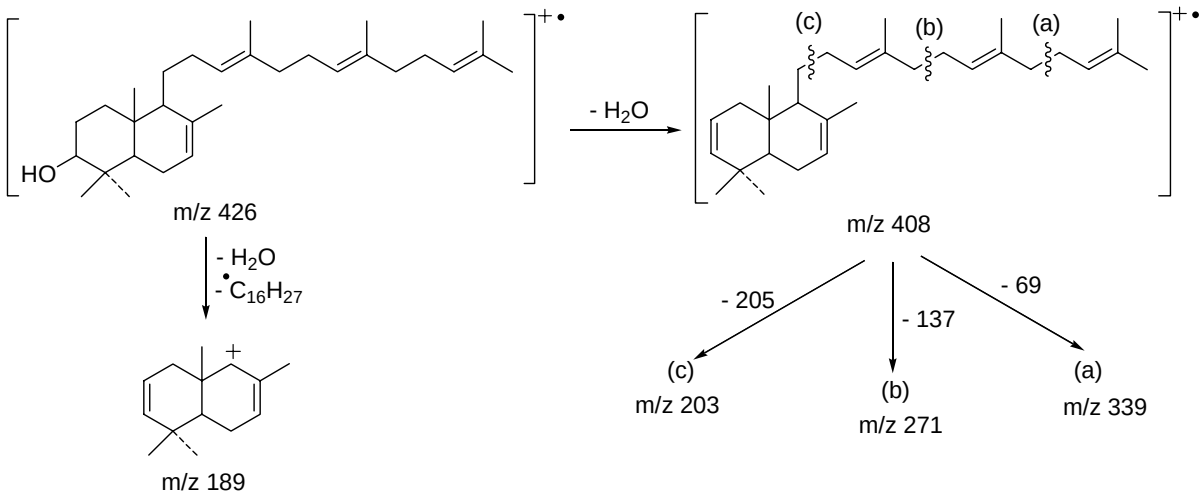
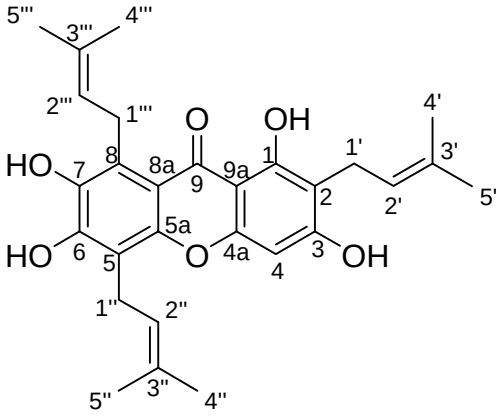


Figure 4.10: Fragmentation mechanism of **PG4** (**105**)

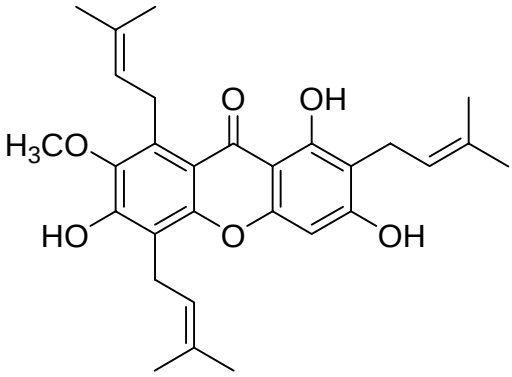
4.3.2.5 Identification of PG5

PG5 was obtained as a yellow powder (3.5 mg) and reacted positively with alcoholic ferric chloride, indicating its phenolic nature. It exhibited a quasimolecular ion $[M+H]^+$ at *m/z* 465.22735, corresponding to the molecular formula $C_{28}H_{33}O_6$ (calcd. 465.22717), and therefore establishing a molecular formula of $C_{28}H_{32}O_6$ which corresponds to 13 double bond equivalents. The ^1H NMR and ^{13}C NMR spectra (see Table 4.7) of **PG5** also displayed signals suggesting a polyhydroxylated polyprenylated xanthone structure (Likhitwitayawuid et al., 1997; Pattalung et al., 1994; Lee and Chan, 1977; Sakai et al., 1993). In fact, its ^1H NMR spectrum showed in the downfield region one aromatic proton resonance at δ 6.47 (s, H-4), and a signal for a chelated OH at δ 13.92 (s, 1-OH). Three prenyl groups were present in the structure of **PG5**, as evident from the following resonances: (a) three olefinic protons at δ 5.24-5.28 (m, H-2', H-2'' and H-2'''); three pairs of methylene protons at δ 3.35 (d, J = 7.1 Hz, H-1'), δ 3.60 (d, J = 7.1 Hz, H-1'') and δ 4.20 (d, J = 6.8 Hz, H-1'''); and six methyl protons at δ 1.63 (br s, H_3-5'''), 1.65 (br s, H_3-5', H_3-5''), 1.78 (br s, H_3-4'), 1.81 (br s, H_3-4'''), 1.88 (br s, H_3-4''). ^1H and ^{13}C NMR spectra of **PG5** both possessed very close resemblance to those of garcinone E (Sakai et al., 1993) and 7-O-methylgarcinone E (**107**) (Likhitwitayawuid et al., 1997) (see Table 3.7).

Finally the identity of **PG5** as garcinone E, 1,3,6,7-tetrahydroxy-2,5,8-triprenylxanthone (**106**), was established by direct comparison of the IR, EIMS, ^{1}H NMR and ^{13}C NMR data with those reported in the literature (Sakai et al., 1993; Likhitwitayawuid et al., 1997).



106



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4.3.2.6 Identification of PG6

PG6 was obtained as a yellow gum and reacted positively with alcoholic ferric chloride, indicating its phenolic nature. It exhibited a molecular ion [M^{+}] at *m/z* 478.23545, leading to the molecular formula $C_{24}H_{26}O_6$ (calcd. 478.23554), and corresponds to 13 double bonds equivalent. The ^{1}H NMR spectrum of **PG6** also displayed signals suggesting a polyhydroxylated polyprenylated xanthone structure (Likhitwitayawuid et al., 1997; Pattalung et al., 1994; Lee and Chan, 1977; Sakai et al., 1993). It showed, in the downfield region, two aromatic proton resonances at δ 6.27 (s, H-4) and δ 6.81 (s, H-5), a signal for a chelated OH at δ 13.76 (s). Signals at δ_H 4.08/δ_C 26.58 (C-1''), 5.24/123.25 (C-2''), 1.99/39.69 (C-4''), 1.80/16.47 (C-5''), 1.99/26.50 (C-6''), 5.00/124.29 (C-7''), 1.58/25.58 (C-9''), 1.52/17.64 (C-10''), δ_C 135.59 (C-3'') and 131.25 (C-8'') were found to be due to the presence of a geranyl group, by comparison of the NMR data with the literature values (Sia et al., 1995; Seo et al., 2002), whereas those at δ_H 3.44/δ_C 21.45 (C-1'), 5.24/121.50 (C-2'), 1.82/17.89 (C-4'), 1.74/25.82 (C-5') and δ_C135.50 (C-3') were due to the presence of a prenyl group; a signal of a methoxy group appeared at δ_H 3.78/δ_C 62.01. In fact, the ^{1}H NMR data of PG6 possessed very close resemblance to those of cowanin (1,3,6-trihydroxy–2-isoprenyl-8-geranylxanthone (Pattalung et al., 1994) (see Table 4.8). Like for **PG1**, the location of the two side chains at C-2, C-8 and the methoxy group at C-7 were

supported by the HMBC correlations of H-1'/C-2, H-1'/C-1, H-1'/C-3. The geranyl group was located at C-8 by the HMBC correlations of H-1''/C-8 (two bonds), H-1''/C-8a (three bonds) and H-1''/C-7 (three bonds). The HMBC correlation from C-7 to the methoxy protons also indicated the locations of the prenyl group at C-8, and the metoxy group at C-7. Hence, **PG6** was identified as 1,3,6-trihydroxy–2-isoprenyl-7-methoxy-8-geranylxanthone, known as cowanin (**108**).

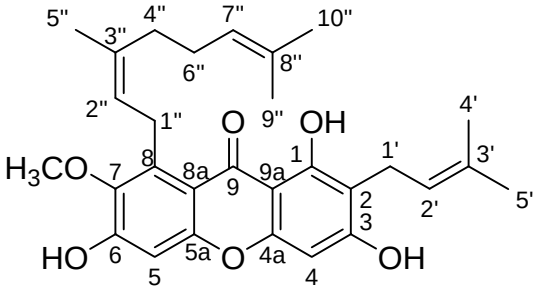

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Table 4.7: ^{13}C NMR data of **PG1** (**103**), **PG2** (**104**) and **PG5** (**106**) and 7-O-methylgarcinone (**107**)

	103	**104**		**106**	**106**[a]	**107**[a]
Atom No	^{13}C NMR (CD_3OD)	^{13}C NMR (CD_3OD)	Atom No	^{13}C NMR $(CD)_3CO$	^{13}C NMR ($CDCl_3$)	^{13}C NMR ($CDCl_3$)
1	161.58	164.78	1	161.63	160.51	160.5
2	111.42	98.80	2	110.78	108.18	108.3
3	163.57	165.92	3	162.93	161.51	161.5
4	93.15	94.09	4	93.10	93.16	93.2
4a	156.16	158.05	4a	155.74	155.08	155.0
5	102.76	102.09	5	114.27	113.30	113.9
5a	156.67	158.41	5a	151.75	151.26	153.5
6	157.79	156.80	6	148.81	148.72	152.3
7	144.73	144.93	7	140.49	139.26	142.2
8	112.23	112.21	8	124.48	124.60	131.8
8a	138.48	138.67	8a	111.82	111.30	111.9
9	183.11	183.05	9	183.32	183.05	182.4
9a	103.77	103.99	9a	103.66	103.65	103.6
OCH_3	61.34	61.41	OCH_3			62.0
1’	22.24	27.03	1’	22.05	21.45	21.4
2’	123.94	125.27	2’	123.58	121.52	121.5
3’	131.74	135.45	3’	131.30	133.66	135.7
4’	26.01	40.82	4’	25.90	24.77	25.8
5’	18.31	16.66	5’	17.91	17.90	17.9
6’		27.61	1’’	23.20	22.56	22.6
7’		125.51	2’’	122.60	121.02	121.1
8’		131.99	3’’	132.36	135.13	132.6
9’		25.80	4’’	25.90	25.83	25.8
10’		17.73	5’’	18.15	17.98	17.9
1’’	27.15		1’’’	25.90	21.45	26.4
2’’	125.19		2’’’	123.61	121.52	123.5
3’’	131.64		3’’’	131.51	133.66	133.9
4’’	17.96		4’’’	25.97	25.77	25.8
5’’	26.01		5’’’	18.32	17.90	18.1

[a] NMR data obtained from the literature (Sakai et al., 1993; Likhitwitayawuid et al., 1997)

Table 4.8: NMR data of **PG6** (**108**) and cowanin(**108**[a])

	108		**108**[a]
Atom No	^{1}H NMR ($CDCl_3$)	^{13}C NMR ($CDCl_3$)	^{1}H NMR ($CDCl_3$)
1		160.66	
2		108.50	
3		161.63	
4	6.27, s	93.25	6.30, s
4a		155.08	
5	6.81, s	101.53	6.86, s
5a		154.59	
6		155.79	
7		142.30	
8		112.24	
8a		137.15	
9		182.00	
9a		103.63	
OCH_3	3.78, s	62.01	3.80, s
1’	3.44, d	21.45	4.45, br d
2’	5,24, m	121.50	5.28, m
3’		135.50	
4’	1.82, s	17.89	1.82, s
5’	1.74, s	25.82	1.76, s
1’’	4.08, d	26.58	4.09, d
2’’	5.24, m	123.25	5.28, m
3’’		135.59	
4’’	1.99, m	39.69	2.03, m
5’’	1.80, s	16.47	1.84, s
6’’	1.99, m	26.50	2.03, m
7’’	5.00, m	124.29	5.03, m
8’’		131.25	
9’’	1.58, s	25.58	1.59, s
10’’	1.52, s	17.64	1.54, s
OH-1	13.76		13.80

[a] NMR data obtained from the literature (Pattalung et al., 1994).

4.4 Biological properties of some compounds isolated from the three plants

4.4.1 Antibacterial activity

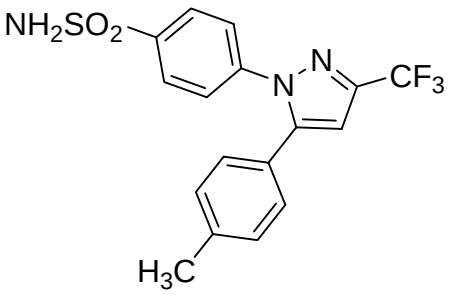
The new triterpene (**ME1**) (**85**) displayed moderate activity against *Bacillus subtilis* ATCC 6633 in concentration > 50 μg/mL. It was inactive against all the other organisms tested.

4.4.2 Antiinflammatory activity

Inflammation is the first response of the immune system to infection and irritation, consisting of certain observable findings; redness, heat, swelling, pain and dysfunction of the organs involved. The modern medicine has developed numerous antiinflammatory or analgesic drugs which may relieve the inflammation symptoms. These include salicylic acid, aspirin, indomethacin, meclofenamic acid, mefenaamic acid, diffusinal, dichlofenac and ibuprofene.

The antiinflammatory activity of seven compounds isolated from the three plants was evaluated. **ME5** (**88**), isolated from *M. eminii* displayed high inhibitory activity (IC_{50} 9.5 μM) on 3α-hydroxysteroid dehydrogenase (3α –HSD), while **ME4** (**89**) and **ME6** (**90**) showed very low activity (IC_{50} 62 and 100 μM, respectively). 3α -HSD appears to be a multifunctional enzyme. Its traditional role is to catalyze early steps in androgen and cortisone metabolism, by reducing a variety of 3-ketosteroids to their corresponding 3α-hydroxysteroids. This enzyme is potently inhibited by the major classes of nonsteroidal and steroidal antiinflammatory drugs (Penning, 1985). High correlations exist between the logarithm of the concentration of drug required to produce 50% inhibition of the enzyme (log IC_{50} value) with that dose in humans required to produce an antiinflammatory response. Thes observations led to the suggestion that the inhibition of the enzyme (3α –HSD), could be used to predict antiinflammatory drug potency (Penning, 1985). The inhibitory activity of **PG2** (**104**) (IC_{50} 14.6 μM) was nearly equal to that of the antiinflammatory indomethacin (IC_{50} 15.0 μM), which was chosen as reference, while the activity for **PG4** (**105**) (IC_{50} 11.7 μM) was higher than that of the refence. **PG4** was therefore more active than the pharmaceutical product indomethacin. The antiinflammatory activity of **PG1** (**103**) was unsignificant (IC_{50} 195 μM).

In addition, **PG2** (**104**) also selectively inhibited the cyclooxygenase-2 (COX-2), also named prostaglandin endoperoxide H synthase-2 (PGHS-2) (IC_{50} 6 μM), which is an isoform of COX-1. COX-1 is considered to be constituvely expressed and to be important for homeostatic functions such as maintaining the integrity of the gastric mucosa, regulating renal blood flow and mediating normal platelet function. The COX-2 isoform, on the other hand, is induced by inflammatory mediators, such as cytokines, in different inflammatory states (Vane and Botting, 1998). Cyclooxygenase enzymes (COXs) contain both cyclooxygenase and peroxidase activities. They catalyze the bis-dioxygenation of arachidonic acid to provide prostaglandin (PG) H_2, the precursor to the primary PGs and thromboxanes (TXs) as illustrated in Figure 4.9 (Hambert and Samuelsson, 1973). The therapeutic efficacy of nonsteroidal antiinflammatory drugs (NSAIDs) derives from their inhibition of COX (Vane, 1971). These compounds, which are widely used in the treatment of pain and inflammation (Lombardino, 1985), non selectively inhibit the two isoforms of cyclooxygenase and thus prevent the upregulation of prostaglandin formation, which otherwise lead to an increase of vascular permeability, edema, hyperalgesia, pyrexia and inflammation (Vane and Botting, 1998; Cryer and Feldman, 1998; Dubois et al., 1998; Barbey et al., 2002). The selective inhibitors may relieve the symptoms in these pathologies while exhibiting a safer toxicity profile (Battistini et al., 1994; Vane, 1988; Vane et al. 1998; Lipsky, 1999). The link between COX-2 activity and inflammation has been dramatically confirmed by the clinical efficacy of two inflammatory, selective COX-2 inhibitors, celecoxib (**109**) and rofecoxib (**110**) (Kozak et al., 2002). Recently, it was demonstrated that some COX-2 inhibitors like Vioxx™ have serious side effects and have been withdrawn from the market.



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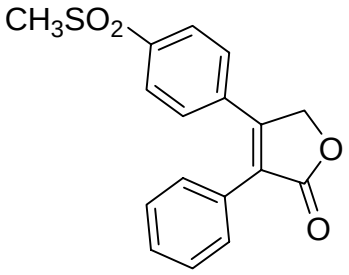

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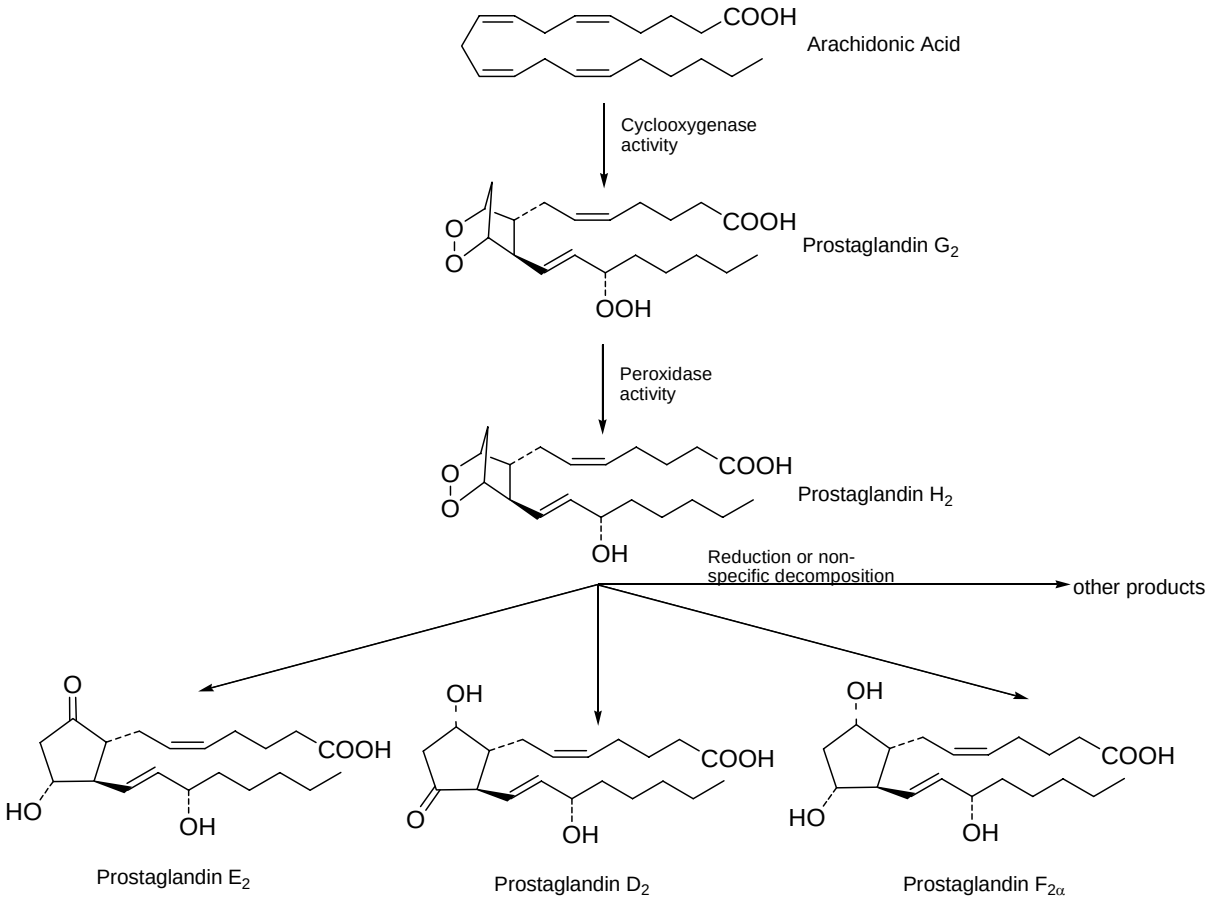
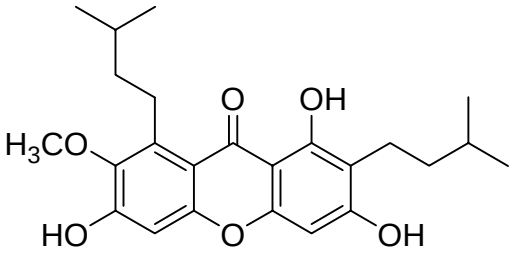


Figure 4.9: Conversion of arachidonic acid.

PG2 (**104**), which has the same selective inhibitory activity like indomethacin (IC_{50} 6 μM) against COX-2 was therefore the most active compound isolated from *Pentadesma grandifolia***. ME5** (**88**) which showed a good activity against 3α-hydroxysteroid dehydrogenase, was inactive against COX-2.

From the point of view of structure-activity relationships, both **PG1** (**103**) and **PG2** (**104**) have a similar oxidation pattern (both are 1,3,6-trihydroxy-7-methoxyxanthones). Structural differences between these two compounds are the pattern of substitution and the type of alkyl chain. **PG2** has a geranyl chain at C-8 and no substitution at C-2. **PG1** on the other hand, is substituted by two isoprenyl chains at C-2 and C-8. Its is considered that the geranyl chain at C-8 plays an important role in the antiinflammatory activity. Both **PG1** and **PG2** inhibited the growth of methicillin-resistant *Staphylococcus aureus* (MRSA). **PG1** had a minimum inhibitory concentration (MIC) of 3.9-30.5 μM, while **PG2** had the highest activity (MIC = 0.7-3.1 μM) (Iinuma et al., 1995). When these compounds were converted to their tetrahydro derivatives by reduction (**111** and **112**), the anti-MRSA

activity was reduced, especially for **112** (Iinuma et al., 1995). These findings suggested that double bonds on the alkyl chains are essential to the activity of a 1,3,6-trihydroxy-7-methoxyxanthone derivative (Iinuma et al., 1995).



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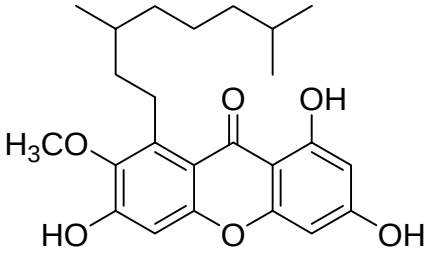

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4.4.3 Antioxidant activity

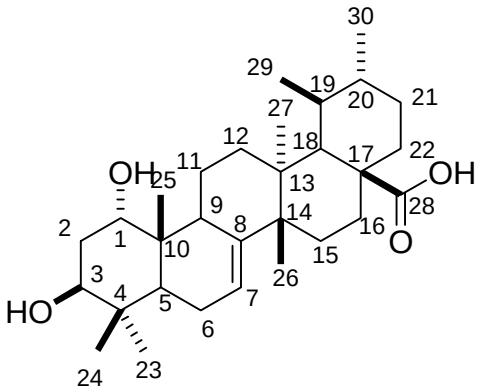
The role of active oxygen species (ROS) and free radicals in the onset of several diseases is increasingly recognized (Halliwell and Gutteride, 1985). Free radical reactions have been implicated in pathology of many human diseases including cancer, ischemic heart disease, aging process, inflammation, diabetes, immunodepression, neurodegradative condition, arteriosclerosis, arthritis and other disease condition (Maxwell, 1995) and have been correlated with oxidative damage. Active oxigen species (ROS) such as superoxide ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\cdot}$) or singlet oxygen ($^1O2$), are products of normal metabolism and are capable of attacking biological molecules leading to injury (Devkota, 2005). When the natural antioxidant protection is unbalanced by exogenous factors such as smoking, ionizing radiations, certain pollutants, organic solvents and pesticides, and endogenous factors such as normal aerobic respiration stimulated polymophonuclear leukocyte and macrophages, onset of disease occurs (Gulcin et al., 2004).

None of these compounds showed anti-oxidant activity when tested against horseradish peroxidase ($IC_{50} \geq 56$ µM).

5 Conclusion and outlook

The aim of this thesis was to isolate and identify secondary metabolites from *Maesopsis eminii* (Rhamnaceae), *Autranella congolensis* (Sapotaceae) and *Pentadesma grandifolia* (Guttiferae) and to establish a relationship between the therapeutic properties of these plants in traditional medicine and their constituents.

The phytochemical study of *Maesopsis eminii* led to the isolation of 13 compounds occurring in the free form as well as in the forms of ether, ester and glycoside, and belonging to three chemical classes: terpenoids, steroids and phenolics. Triterpenoids were reported here for the first time to occur in this species and one of them (**85**) was a previously unknown compound. This new triterpene (**85**) displayed moderate activity against *Bacillus subtilis* ATCC while 5-hydroxydigitolutein **(88**) displayed a good anti-inflammatory activity. A mixture of (+)-catechin and (-)-epicatechin (**97**+**98**) was isolated and (+)-catechin (**97**) was described as an anticancer agent. The fact that these compounds isolated from *Maesopsis eminii* belong to several groups of natural products presenting various biological properties could explain the wide use of this plant in traditional medicine, thanks to the diuretic, purgative, emetic and antidiarrhoetic activity of its bark.



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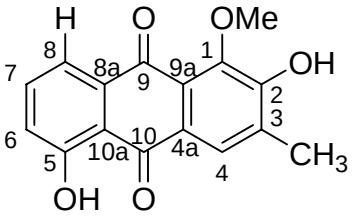

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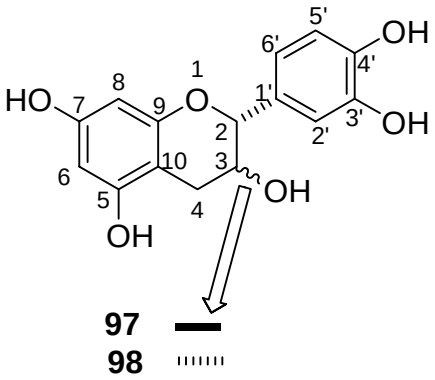

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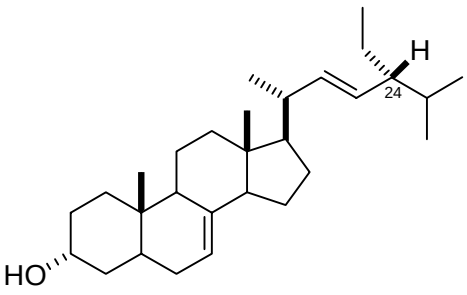

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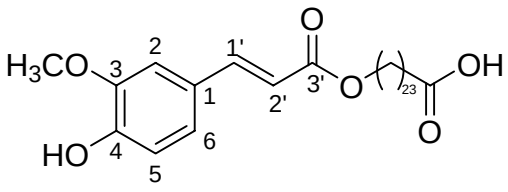
Chemical investigation of *Autranella congolensis* led to the isolation of six compounds of which three were pentacylic triterpenes of the taraxerane type, together with one Δ^7-sterol (24*R*)-stigmast-7,22(*E*)-dien-3α-ol (chondrillasterol, **26**), and two phenolic compounds (+)-catechin (**97**) and 24-feruloytetracosanoic acid (**102**). All these compounds were previously known. Their structures have been elucidated and verified by spectroscopic methods. Taraxerone (**100**) and Δ^7-sterol were previously reported as constituents of the barks of the *Mimusops* species. With respect to chemotaxonomy, this similarity of the constituents of *Autranella congolensis* which is studied here for the first time with those of the genus *Mimusops* confirms its membership of the family Sapotaceae. The use of this plant as anti-inflammatory agent could be linked to the presence of (+)-catechin (**97**) and other constituents, that could not be isolated in pure form. The polar extract was a very complex mixture and since many of the plant secondary metabolites are produced in response to infection (phytoalexins) in small quantities, isolation is sometimes difficult.



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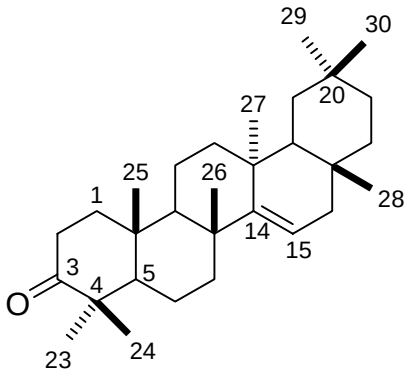
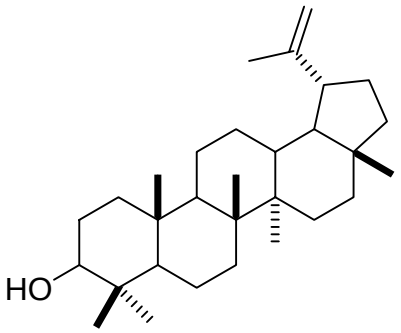


100

Six known compounds have also been isolated from the root bark of *Pentadesma grandifolia*, a bicyclic triterpenoid (13*E*,17*E*)-polypoda-7,13,17,21-tetraen-3β-ol (**105**),

lupeol (**23**) and four xanthones α-mangostin (**103**), rubraxanthone (**104**), garcinone E (**106**) and cowanin (**108**). Compounds **104** and **105** displayed good anti-inflammatory activity and **104** was found to selectively inhibit the cyclooxygenase-2 (COX-2). Lupeol (**23**) exhibited hypotensive activity (Harbone and Baxter, 1993), in vitro antimalarial activity (Alves et al., 1997), as well as anti-inflammatory activity (Geetha and Varalakshmi, 1988).



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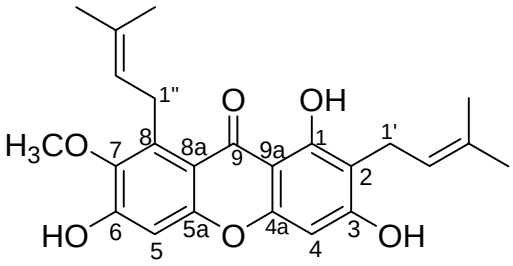

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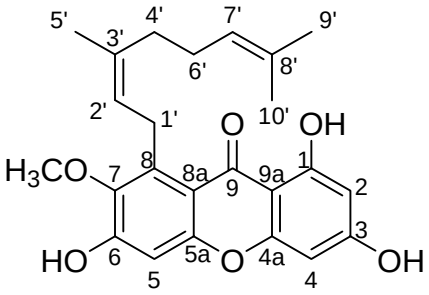

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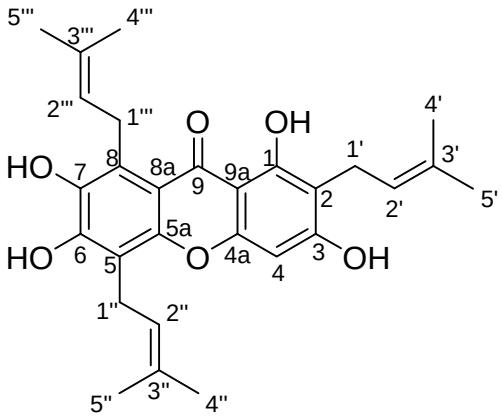

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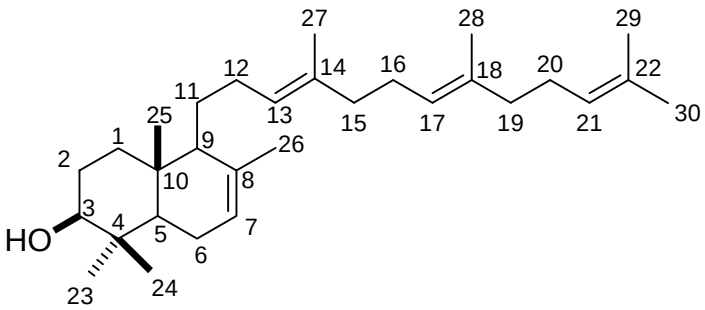

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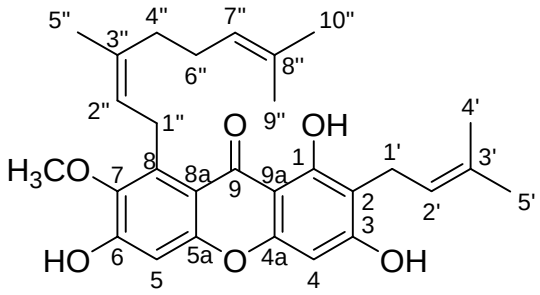

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These various biological properties of compounds isolated from the root bark of *Pentadesma grandifolia* would be at the origin of its ethnomedicinal use. The absence of any sterol in the root bark of *Pentadesma grandifolia* contrast with the widespread occurrence of phytosterol in the bark and timber of higher plants, including plants of the family Guttiferae. We therefore intend to extend the investigation to other parts of this species in order to confirm or infirm the absence of sterol in this plant in one hand and eventually to isolate some further constituents on the second hand.

Taking into consideration what precedes, it is necessary to affirm that plants remain an untapped reservoir of potentially useful chemical compounds as drugs, as unique templates that could serve as starting material for synthetic chemistry, or as tools for understanding the biological processes better (Farnsworth, 1984). However, in light of the alarming loss of plant diversity around the world due to habitat destruction and unsustainable havesting practices, some of the medicinal plants with interesting lead compounds will disappear. One should therefore recognize the importance of the conservation of natural resources and sustainable exploitation of such resources for the overall public benefit.

6 Experimental Part

6.1 Instruments and Materials

6.1.1 Instruments

6.1.1.1 General Methods

Melting point apparatus:

Melting points were determined on a Büchi B-540 melting point apparatus and are uncorrected.

NMR Spectroscopy:

The ^{1}H NMR spectra were recorded at 250 MHz, 500 MHz or 600 MHz and ^{13}C NMR spectra were recorded at 63 MHz, 126 MHz and 151 MHz, respectively, using deuterated solvents, with tetramethylsilane (TMS) as an internal standard. The spectrometers were of the type:

-Bruker AC 250 P (^{1}H NMR, 250 MHz and ^{13}C NMR, 63 MHz)

-Bruker AM Avance DRX 500 (^{1}H NMR, 500 MHz and ^{13}C NMR, 126 MHz)

-Bruker Avance 600 (^{1}H NMR, 600.1 MHz and ^{13}C NMR, 151 MHz).

IR Spectroscopy:

IR spectra (KBr tablet or film) were recorded on a Jasco FT/IR-410 spectrometer, at room temperature.

MS:

EI: VG Autospec X (Micromass Co); Electron Impact 70 eV

CI: VG Autospec X (Micromass Co); Chemical Ionization 45 eV

ESI: Esquire 3000 Electrospray Ionization (Bruker Daltonik Co)

FT-ICR-MS: APEX III (Bruker Daltonik Co)

TLC:

Silica gel 60 F_{254}, 0.1 mm thick (Merck) and RP-18 F_{254}S, 0.25 mm thick (Merck) on the form of aluminum sheets or glass plates were used as stationary phase. The TLC plates were developed in various solvent systems at room temperature. All spots were detected by

fluorescence (254 nm) and/or with a solution of Molybdate/Ce^{4+} reagent or a diluted solution of sulfuric acid (50% v/v) in MeOH followed by heating.

PTLC:

The preparative TLC plates consisted of a 1 mm thick layer of Silica gel 60 F_{254} with a fluorescent indicator bonded to glass support (Merck). The preparative TLC plate sizes 20 x 20 cm provided a large area for chromatographic separation. After the separation, the plate was dried and the products were detected with a UV lamp at 254 nm. The bands were detached from the plate and extracted several 25 mL of CH_2Cl_2-MeOH 9:1 or EtOAc-MeOH 9:1, depending on the solubility of the product. The extract was finally concentrated to dryness to give the desired product.

Table 6.1: Molybdate/Ce^{4+} reagent

	Mass content / kg of solution
$(NH_4)_6Mo_7O_{24}.4H_2O$	50 g (5.0%)
$Ce(SO_4)_2$	2 g (0.2%)
H_2SO_4 (conc.)	50 g (27 mL) (5.0%)
Water	898 g (898 mL) (89.8%)

The Liebermann - Burchard test was used to detect terpenoids and steroids while the Shinoda test was used to detect flavonoids. Iron (III) chloride test was used for phenolic compounds.

Liebermann - Burchard test: Unsaturated and hydroxylated triterpenes and steroids show a red, blue or green coloration with acetic anhydride and sulfuric acid (Abisch and Reichstein, 1960). Since terpenoids or their saponins tend to produce a pink or purple shade and steroids and their saponins a blue-green coloration, differentiation of the two classes is possible.

Iron (III) chloride test: Phenolic compounds show a red, blue, green or purple coloration with 1% aqueous iron (III) chloride.

Shinoda test: Flavonoids show effervescence and color change with conc. HCl and magnesium turnings. The coloration can be violet or red-brown.

Flash chromatography and atmospheric pressure column chromatography:

Vertical glass columns of different sizes were usued. A vertical glass column was filled with with Silica gel 60, 40-63 μm (Merck) and the sample to be separated was placed on top of this support. The rest of the column was filled with a solvent which, under the influence of gravity, moves the sample through the column.

In the case of flash column chromatography, the solvent was driven through the column by applying positive pressure. When applying positive pressure on top of the column, most separations could be performed in less than 20 minutes with improved separations compared to the traditional column chromatography. This makes flash column chromatography the method of choice for most organic chemists when purifying organic compounds.

Analytical HPLC

Software	Chromquest
Autosampler	Thermo Separation Products AS 100
Pump	Thermo Separation Products P 4000
Controller	Thermo Separation Products SN 4000
Detector	UV detector-Thermo Separation Products UV 6000
Columns	Vydac^{TM} 300 C_{18}, 5 μm, 250x4 mm,
	Phenomenex, Jupiter C_{18}, 5 μm, 250x4.6 mm
	Vydac^{TM} 218TP54 efficiency C_{18}, 5 μm, 250x4.6 mm
Eluent A	95% H_2O, 5% ACN, 0.1% TFA
Eluent B	95% ACN, 5% H_2O, 0.1% TFA

Methods used for analytical HPLC:

Table 6.2: The gradient for analytical method 1

Time	Eluent A (%)	Eluent B (%)	Flow (mL/min)	UV (nm)
0	100	0		
45	0	100		
50	0	100	1	220 and 254
55	100	0		

Table 6.3: The gradient for analytical method 2

Time	Eluent A (%)	Eluent B (%)	Flow (mL/min)	UV (nm)
0	100	0		
5	100	0		
30	0	100	1	220 and 254
40	0	100		
50	100	0		
55	100	0		

Preparative HPLC

Software	Chromquest
Pump	Thermo Separation Products P 4000
Controller	Thermo Separation Products SN 4000
Detector	Thermo Separation Products UV 1000
Columns	Vydac^{TM} 300, C_{18}, 10 µm, 250x220 mm
	Vydac^{TM} 218 TP 1022 efficiency, C_{18}, 250x220 mm
Eluent A	95% H_2O: 5% ACN: 0.1% TFA
Eluent B	95% ACN: 5% H_2O: 0.1% TFA

Methods used for Preparative HPLC

Table 6.4: Preparative method 1

Time	Eluent A (%)	Eluent B (%)	Flow (mL/min)	UV (nm)
0	100	0		
45	0	100		
50	0	100	10	254
55	100	0		

Table 6.5: Preparative method 2

Time	Eluent A (%)	Eluent B (%)	Flow (mL/min)	UV (nm)
0	100	0		
5	100	0		
30	0	100	10	254
40	0	100		
50	100	0		
55	100	0		

Sonification Baths

Sonorex RK 510 H (Bandelin)

Sonorex TK 52 (Bandelin)

Solvents

All solvents used were previously distilled separately except those which were obtained from the compagny Merk. Methylene chloride was first distilled over calcium chloride and finally from calcium hydride. Ethyl acetate and Petrolether were distilled from calcium chloride. Tetrahydrofuran was first distilled from calcium chloride and finally from calcium hidryde and sodium. Water was purified using a Millipore water purification system. Acetonitrile (gradient grade) was obtained from Merck. Trifluoroacetic acid was obtained from Solvay Fluor and Derivative GmbH.

6.1.1.2 Antimicrobial activity screening

Antimicrobial studies was carried out by Mr. Hilaire V. Kemami Wangun, from the Hans-Knöll-Institut for Natural Product Research, Jena, Germany.

6.1.1.2.1 Materials

Inoculated cultures of indicator organisms (bacteria, fungi…)
Nutrient agar plate
Filter-paper discs
Solutions of different concentrations of the test compounds

6.1.1.2.2 Procedure

A commonly used agar diffusion procedure that measures the antimicrobial activity was used. The culture media are inoculated using aseptic techniques with the selected culture by spreading it across the agar surface. Filter-paper discs containing known concentrations of different antimicrobial agents are then placed directly onto the inoculated agar. After incubation at 37 °C for 24 to 48 hours, the plates are examined for any growth inhibition, meaning that the microbe is susceptible to the agent present. If there is no or very little inhibition, then the compound is not active against the microbe. The size of the zones of inhibition is then recorded; the diameter of the zone is proportional to the amount of antimicrobial agent, the solubility of the agent, the diffusion coefficient, and the overall effectiveness of the agent. Zones observed on the plates are then compared to standard data to determine if the agent can be considered as really active against the test organism. The microorganisms used for the test were:
Bacteria: *Bacillus subtilis ATC 6633, Eschericia coli*.
Yeasts: *Saccharomyces cerevisiae, Rhodotorula rubra IMET 25030, Sporobolomyces salmonicolor SBUG 549, Kluyveromyces marxianus IMET 25148, Candida formata IMET 25000*.
Fungi: *Fusarium culmorum JP 15, Penicillium notatum JP36, Glomerella cingulata, Penicillium sp, Phoma destructiva I 1015*.

6.1.1.3 Antiinflammatory activity screening

The antiinflammatory studies were carried out by Dr. Albert Härtl from the Leibniz-Institute for Natural Product Research and Infection Biology, Hans-Knöll-Institut, Jena, Germany.

6.1.1.3.1 Inhibition of 3α-hydroxysteroid dehydrogenase

Antiinflammatory activity of the purified compounds was evaluated using the protocol described in the literature by Penning (1985). This method consists of monitoring the reduction of 3-ketosteroid, e.g., 5β-dihydrocortisone (5β-pregnan-17α,21-diol-3,11,20-trione), catalyzed by the NAD(P)-linked 3α-hydroxysteroid dehydrogenase, to its corresponding 3α-hydroxysteroid.

Enzyme Assays: The reduction of 5β-dihydrocortisone was monitored by measuring the change in the absorbance of the pyridine nucleotide at 340 nm. Each essay (1 mL) contained the following: 0.840 mL H_2O, 0.100 mL 1 M potassium phosphate buffer (pH 6), 20 µl 9 mM 5β-dihydrocortisone, and 30 µl acetonitrile. The reactions were initiated by the addition of enzyme (0.6 µg), and the optical density change was followed over a time course of 5 min. Control experiments in which the cytosol was added to incubations in which either the 5β-dihydrocortisone or NADPH was absent indicated that the presence of both substrates was required before the cytosol would promote a change in absorbance at 340 nm (Penning, 1985).

Inhibition Studies: Increasing amounts of steroidal and non steroidal antiinflammatory drugs, added to 3α-hydroxysteroid dehydrogenase of rat liver cytosol, inhibit the reduction of 5β-dihydrocortisone (5β-pregnan-17α,21-diol-3,11,20-trione) to its corresponding 3α-hydroxysteroid. The degree of inhibition was used to predict the pharmacological potency of the drugs and the concentration of drug required to reduce the rate of 5β-dihydrocortisone reduction by 50% (IC_{50} value) was computed from the resulting dose-response curves. This NAD(P)-linked enzyme therefore plays an important role in both androgen and cortisone metabolism (Holt and Scriefers, 1973; Tomkins, 1956; Tomkins, 1956a). It should be noted that cortisone is an hormone useful in suppressing of inflammation (Penning, 1985).

6.1.1.3.2 Inhibition of cyclooxygenase-2 (COX-2)

The Cayman Chemical Chemiluminescent COX (ovine) Inhibitor Screening Assay was used to evaluate the inhibitory activity of the isolated compounds. This assay utilizes the

heme-catalysed hydroperoxidase activity of ovine cyclooxygenases to generate luminescence in the presence of a cyclic naphthalene hydrazide and the substrate arachidonic acid (Forghani et al., 1998). Arachidonate-induced luminescence was shown to be an index of real-time catalytic activity and demonstrate the turnover activation of the enzyme (Forghani et al., 1998). Inhibition of cyclooxygenase activity measured by luminesence, by a variety of selective and non-selective inhibitors showed potencies similar to those observed with other *in vitro* and whole cell methods (Forghani et al., 1998). This Cayman Chemical Chemiluminescent COX assay was described as a time saving tool for screening vast numbers of inhibitors.

Preparation of the reagents

3 ml of assay buffer concentrate was diluted with 27 ml of HPLC-grade water and the final assay buffer (0.1 M Tris-HCl, pH 8) was used for dilution of heme and arachidonic acid prior to asaying. 58 µl of heme (dissolved in DMSO) was diluted with 942 µl of dilute assay buffer. To avoid repeated freezing and thawing, the solution of COX was aliquoted into several small vials and stored at –80 °C. 30 µl of enzyme was diluted with 570 µl of dilute assay buffer and store on ice. For each well 10 µl of enzyme was needed. A solution of arachidonic acid in ethanol was prepared and stored at –80 °C when not beeing used. 100 µl of the substrate was transferred to another vial in which 100 µl of 0.1 M KOH was added and dilute with 9.8 ml of Assay Buffer (dilute) to achieve a final concentration of 116 µM. The prepared arachidonic acid solution was used within 1 hour. A 50 µl aliquot yielded a final concentration of 20 µM in the wells. The chemiluminescent substrate was a solution of naphthalene hydrazide which was then used as supplied.

Performing the assay

The final volume of the assay was 290 µl in all the wells and the assay was performed at 25 °C. The COX sample were assayed in triplicate in 96-well microplate. The Luminometer dispensed both the chemiluminescent substrate and the arachidonic acid (a Luminometer with two syringes).

10 µl of heme, 10 µl of Assay Buffer, and 10 µl of DMSO were added to three wells which were used as background wells, 10 µl of heme, 10 µl of Enzyme (either COX-1 or COX-2), and 10 µl of DMSO were added to three other wells and used as 100% initial activity wells, while inhibitor wells (three wells) contained a mixture of 10 µl of heme, 10 µl of

Enzyme (either COX-1 or COX-2), and 10 µl of inhibitor. Inhibitors were dissolved in dimethyl sulfoxide. Several dilutions of the inhibitor were made. The reaction was then initiated by adding 200 µl of assay buffer to all the wells used. The plate was then inserted into the luminometer which was allowed to dispense 10 µl of the chemiluminescent substrate and then immediately 50 µl of arachidonic acid to all the wells used. The luminescent unit was immediately read for 10 seconds per well.

Calculations

The average relative luminescent units (RLU) of all the samples was determined. The RLU of the background wells was subtracted from the RLUs of the 100% initial activity and the inhibitor wells. Each inhibitor sample was also subtracted from 100% initial activity sample, and then divided by the 100% initial activity sample, and finally multiplied by 100 to give the percent inhibition.

The IC_{50} value (concentration at which there was 50% inhibition) was determined either by graphing the Percent Inhibition or Percent Initial Activity by the Inhibitor Concentration.

6.1.1.4 **Antioxidant activity screening**

The antioxidant studies were carried out by Dr. Albert Härtl from the Leibniz-Institute for Natural Product Research and Infection Biology, Hans-Knöll-Institut, Jena, Germany.

DPPH (1,1-Diphenyl-2-picryl hydrazyl) free radical scavenging activity

The reaction mixture containing 5µl of test sample (1mM in DMSO) and 95µl of DPPH (1,1-Diphenyl-2-picryl hydrazyl) (Sigma, 300µM) in ethanol was taken in a 96-well microtiter plate and incubated at 37 °C for 30 min. The absorbance was measured at 515 nm. Percent radical scavenging activity was determined by comparison with a DMSO containing control. BHA (3-t-butyl-4-hydroxyanisole) was used as a positive controle. All the chemicals used were of Analytical Grade (Sigma, USA).

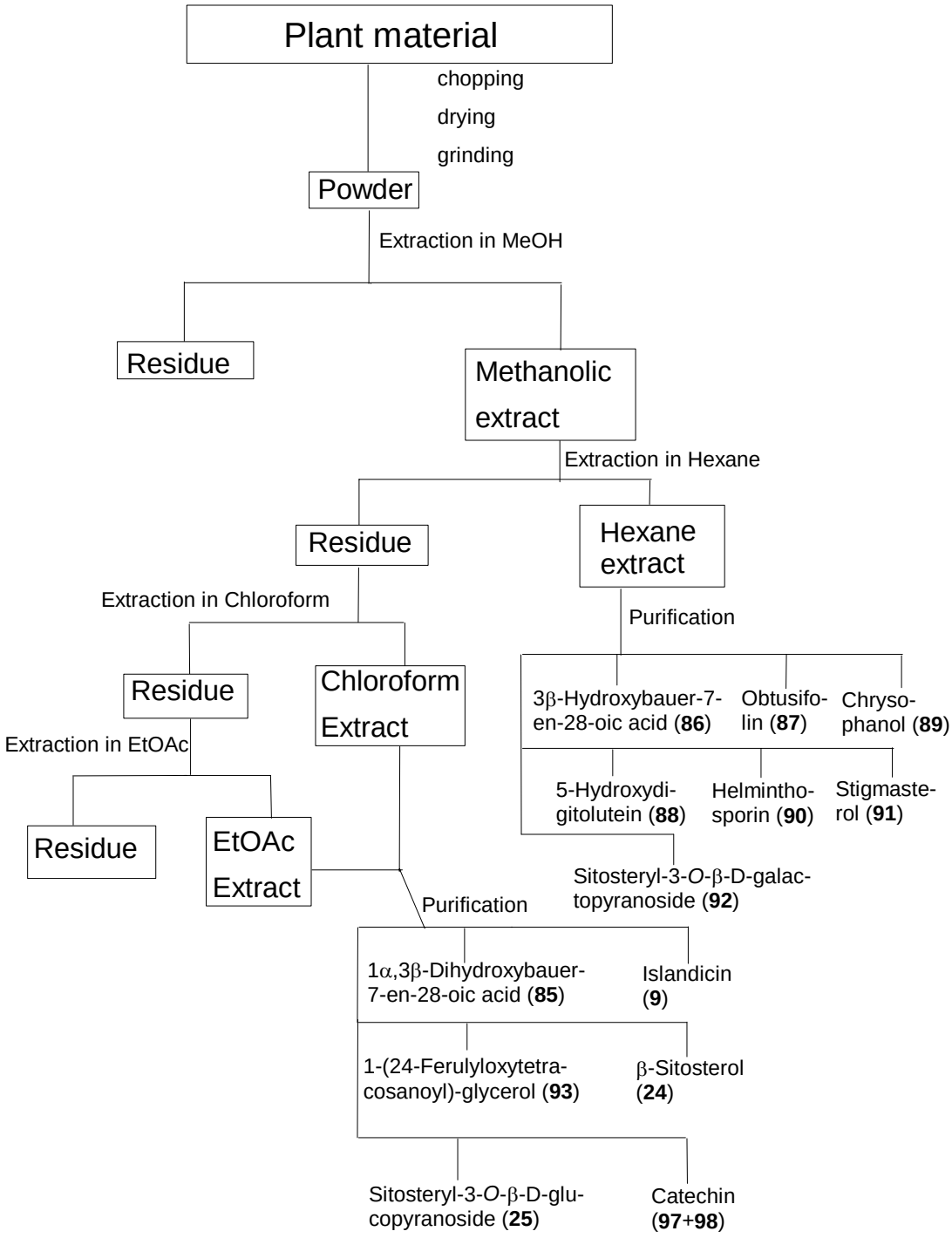
6.1.2 Plant material

6.1.2.1 Maesopsis eminii

The stem bark of *Maesopsis eminii* was collected in February 2001 at Mfou in the Centre Province of Cameroon. The sample was identified at the Cameroon National Herbarium in Yaounde, where a voucher specimen is on deposit under the references RL5561; f 234 /SRF/Cam.

6.1.2.1.1 Extraction

Air-dried, powdered stem bark of *M. eminii* (3 kg) was extracted at room temperature with MeOH and concentrated to dryness to afford a viscous residue (70 g). This residue was then re-extracted with petrol ether, $CHCl_3$ and EtOAc, respectively (see scheme below).



6.1.2.1.2 Separation and purification

The concentrated petrol ether extract, an oily mixture (4 g), was subjected to column chromatography over silica gel and eluted with a petrol ether/EtOAc gradient of increasing polarity, EtOAc and EtOAc/MeOH 9:1, resulting in 130 fractions of 150 mL each which were combined on the basis of TLC analysis. The elution conditions for the chromatographic fractions are given in Table 6.6.

Table 6.6: Chromatographic Fractions of the PE-Extract

Fractions	Eluent
Oily mixture	petrol ether
1-25	petrol ether/EtOAc 19:1
26-36	petrol ether/EtOAc 9:1
37-62	petrol ether/EtOAc 17:3
63-110	petrol ether/EtOAc 4 :1→1:4 and EtOAc
111-130	EtOAc/MeOH 9:1

Fractions 4-15 were eluted with a mixture of petrol ether/EtOAc 19:1, concentrated and chromatographed a second time on HPLC using preparative method 1 to afford compound **ME4** (25 mg) and **ME6** (11 mg) after evaporation of solvent followed by lyophilisation. The two compounds were obtained at the retention time 34 and 38.4 min (preparative HPLC), respectively.

Fractions 29-33 were eluted successively with mixtures of petrol ether/EtOAc 19:1 and petrol ether/EtOAc 9:1, respectively, and evaporated to afford compound **ME7** (60 mg) which crystallized on standing.

Fractions 34-41 were eluted successively with mixtures of petrol ether/EtOAc 9:1 and ether/EtOAc 17:3, respectively, concentrated, and chromatographed a second time over silica gel, eluted with pure CH_2Cl_2 to afford compound **ME5** (40 mg).

Fractions 42-52 were eluted with a mixture of petrol ether/EtOAc 17:3, concentrated, and chromatographed a second time on HPLC using preparative method 1 to afford compound **ME3** (5.5 mg) with a retention time 30.5 min.

Fractions 61-63 were eluted with a mixture of petrol ether/EtOAc 4:1 and evaporated to afford compound **ME2** (9 mg), which crystallized on standing.

Fractions 105-129 were eluted successively with a mixture of petrol ether/EtOAc 1:4 and EtOAc respectively, concentrated, and chromatographed a second time over silica gel, eluted with a mixture of $CHCl_3$-MeOH 9:1 to afford **ME8** (10 mg), sitosterol 3-O-β-D-galactopyranoside.

The combined mixture of the $CHCl_3$ and EtOAc extracts (8 g) was subjected to a column chromatography over silica gel and eluted with a CH_2Cl_2-MeOH gradient of increasing polarity resulting in 140 fractions (Table 6.7) of 20 mL each which were combined on the basis of TLC analysis.

Table 6.7: Chromatographic Fractions of the combined mixture

Fractions	Eluent
1-80	$CHCl_3$-MeOH 97:3
81-110	$CHCl_3$-MeOH 93:7
111-140	$CHCl_3$-MeOH 22:3
	$CHCl_3$-MeOH 4:1

Fractions 1-3 were eluted with a mixture of $CHCl_3$-MeOH 97:3, concentrated, and chromatographed a second time on HPLC using preparative method 1 to afford compound **ME11** (6 mg) with a retention time 45.9 min.

Fractions 4-18 eluted with a mixture of $CHCl_3$-MeOH 97:3, were concentrated and chromatographed a second time over silica gel and eluted with a mixture of CH_2Cl_2-MeOH 99:1 to afford compound **ME12** (β-sitosterol).

Fractions 35-57 were combined to afford a product which was subjected to PTLC for purification using a mixture of $CHCl_3$-Toluene-MeOH 19:4:1; compound **ME9** (70 mg) was obtained.

Fractions 72-92 were eluted with a mixture of $CHCl_3$-MeOH 97:3 and $CHCl_3$-MeOH 93:7, respectively, and combined to afford 250 mg of a product which, after purification by isocratic elution over a small column using a mixture of $CHCl_3$-MeOH 19:1, gave compound **ME1** (100 mg).

Fractions 93-114 were eluted successively with the mixtures of $CHCl_3$-MeOH 93:7 and $CHCl_3$-MeOH 22:3, respectively, concentrated, and chromatographed a second time over silica gel, eluted with a mixture of CH_2Cl_2-MeOH gradient of increasing polarity. Sub-fractions 87-109, eluted with a mixture of CH_2Cl_2-MeOH 37:3 were concentrated to dryness to afford **ME13** (27 mg), sitosterol 3-O-β-D-glucopyranoside.

Fractions 124-136 were eluted with a mixture of $CHCl_3$-MeOH 22:3 and $CHCl_3$-MeOH 4:1, respectively, concentrated, and chromatographed a second time over silica gel, eluted with a mixture of CH_2Cl_2-MeOH 17:3 to afford **ME14** (180 mg) (+ and – catechin).

Acidic hydrolysis of ME8

ME8 (10 mg) in MeOH (4.5 mL) and H_2O (0.5 mL) was refluxed in dilute HCl (1mL) for 6 hours. Then the mixture was cocentrated and H_2O was added, followed by neutralisation with 10% $NaHCO_3$. After extraction with EtOAc, the aglycone which was identified as β–sitosterol by comparison with authentic compound (TLC, R_f 0.42 in $CHCl_3$/MeOH 97:3), was removed. The H_2O phase was evaporated under reduced pressure to yield D-galactose, which was confirmed by comparison (TLC, EtOAc/MeOH/H_2O 7:1:2, R_f 0.13) with a sample of β-D(+)-galactose.

Acetylation of ME9

ME9 (30 mg) was dissolved in C_5H_5N-Ac_2O (4 mL, 1:1) and stirred for 24 hours at room temperature. Crushed ice was added to the mixture, followed by extraction with EtOAc. The EtOAc extract was then evaporated to dryness to yield **ME10**, tetracosanoic acid, 24-[[(2*E*)-3-[4-(acetyloxy)-3-methoxyphenyl]-1-oxo-2-propenyl]oxy]-, 2,3-bis(acetyloxy) propyl ester.

1α,3β-Dihydroxybauer-7-en-28-oic acid (**ME1**) (**85**), $C_{30}H_{48}O_4$;
colorless crystals; **mp** 302-304 °C;
IR $\tilde{\nu}_{max}$ (KBr) cm^{-1}: 3423, 2960, 2930, 2871, 1693, 1457, 1380, 1207, 1041, 790, 651;
^{1}H NMR (600 MHz, DMSO-D_6), see Table 4.1;
^{13}C-NMR (63 MHz, DMSO-D_6), see Table 4.1;
MS (CI, NH_3) *m/z* (rel. int. %) 490 (66.46), 472 (16.33), 456 (25.49), 455 (77.34), 437 (100), 427 (41.40), 391 (23.17), 263 (3.39), 227 (6.24), 207 (16.25), 189 (5.52), 173 (5.86);
HRMS (ESI-FT-ICR) *m/z* calcd.: 471.34798 $[M-H]^-$, found: 471.34805 $[M-H]^-$.

3β-Hydroxy-bauer-7-en-28-oic acid (**ME2**) (**86**), $C_{30}H_{48}O_3$;
colorless powder; **mp** 308-310 °C (Lit. Mersuriyen et al., 1986; 305-308 °C);
^{13}C-NMR (126 MHz, DMSO-D_6), see Table 4.1;
MS (EI) *m/z* (rel. int. %) 456 (69), 441 (100), 438 (6), 423 (54).

Obtusifolin (**ME3**) (**87**), $C_{16}H_{12}O_5$;

yellow powder, **mp** 239-241 °C (Lit. Cameron et al.,1989; 241-243 °C);

IR $\tilde{\nu}_{max}$ (KBr) cm^{-1}: 3355, 1647, 1594, 1466, 1385, 1278, 765, 733;

^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 2.40 (3H, s, Me), 4.00 (3H, s, OMe), 6.76 (1H, s br, exchangeable with D_2O, 2-OH), 7.27 (1H, d, *J*=7.8 Hz, H-7), 7.63 (1H, t, *J*=7.8 Hz, H-6), 7.78 (1H, d, *J*=6.9 Hz, H-5), 7.98 (1H, s, H-4), 12.83 (1H, s, 8-OH);

^{13}C-NMR (126 MHz, $CDCl_3$, δ ppm), see Table 4.2;

HRMS (ESI-FT-ICR) *m/z* calcd.: 285.07539 $[M+H]^+$, 307.05708 $[M+Na]^+$, found: 285.07575 $[M+H]^+$, 307.05769 $[M+Na]^+$;

MS (ESI) *m/z* 590.8 $[2M+Na]^+$, MS (ESI, negative mode) *m/z* 282.9 $[M-H]^-$.

Chrysophanol (**ME4**) (**89**), $C_{15}H_{10}O_4$;

yellow powder, **mp** 171-174 °C (Lit. Ekpa et al., 1985; 194-196°C) ;

IR $\tilde{\nu}_{max}$ (KBr) cm^{-1}: 3429, 2921, 2851, 1672, 1627, 1457, 1384, 1272, 1210, 1055, 753, 727;

^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 2.46 (3H, s, Me), 7.08 (1H, d, *J*=1.2 Hz, H-2), 7.28 (1H, dd, *J*=8.4, 1.0 Hz, H-7), 7.63 (1H, d, *J*=1.2 Hz, H-4), 7.66 (1H, t, *J*=8.0 Hz, H-6), 7.80 (1H, dd, *J*=7.5, 1.0 Hz, H-5), 11.99 (1H, s, 1-OH), 12.10 (1H, s, 8-OH);

^{13}C-NMR (126 MHz, $CDCl_3$, δ ppm), see Table 4.2;

HRMS (CI, NH_3) *m/z* calcd.: 255.06549 $[M+H]^+$, found: 255.06573 $[M+H]^+$;

MS (CI, NH_3) *m/z* 255 $[MH]^+$, 254 $[M]^+$.

5-hydroxydigitolutein (**ME5**) (**88**), $C_{16}H_{12}O_5$;

orange needles, **mp** 235 – 237 °C;

IR $\tilde{\nu}_{max}$ (KBr) cm^{-1}: 3333 (OH), 1633, 1560, 1475, 1315, 1272, 1092, 1038, 749;

^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 2.36 (3H, s, Me), 3.93 (3H, s, OMe), 7.34 (1H, dd, *J*=7.5, 1.3 Hz, H-6), 7.68 (1H, dd, *J*=7.5, 1.3 Hz, H-8), 7.79 (1H, t, *J*=7.9 Hz, H-7), 7.86 (1H, s, H-4), 8.02 (1H, s br, exchangeable with D_2O, 2-OH), 12.83 (1H, s, 5-OH).

^{13}C-NMR (126 MHz, $CDCl_3$, δ ppm), see Table 4.2;

HRMS (ESI-FT-ICR) *m/z* calcd.: 285.07554285 $[MH]^+$, found: 285.07575285 $[MH]^+$;

MS (CI, NH_3) *m/z* 285 $[MH]^+$, 284 $[M]^+$

Helminthosporin (**ME6**) (**90**), $C_{15}H_{10}O_5$;
red powder, **mp** 196-198 °C;
IR $\tilde{\nu}_{max}$ (KBr) cm^{-1}: 3433 (OH), 1602, 1457, 1296, 1260, 1231, and 1181, 805;
^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 2.46 (3H, s, Me), 7.08 (1H, s, H-2), 7.24 (2H, H-6, H-7), 7.66 (1H, s, H-4), 12.10 (1H, s, 1-OH), 12.28 (1H, s, 5-OH), 12.97 (1H, s, 8-OH).
^{13}C-NMR (126 MHz, $CDCl_3$, δ ppm), see Table 4.2;
HRMS (CI, NH_3) *m/z* calcd.: 271.06008 $[MH]^+$, found: 271.06065 $[MH]^+$;
MS (CI, NH_3) *m/z* 271 $[MH]^+$, 255 $[MH-O]^+$

Stigmasterol (**ME7**) (**91**), $C_{29}H_{48}O$;
white powder, **mp** 138-140 °C (Lit. Ikan, 1991; 139-140 °C)
IR $\tilde{\nu}_{max}$ (KBr) cm^{-1}: 3416, 2865, 1463, 1380, 1055, 1022, 958, 838 and 800;
^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 0.53 (3H, s, Me-18), 0.79 (3H, d, *J*=6.45 Hz, Me-26), 0.80 (3H, s, Me-19), 0.83 (3H, t, *J*=6.50 Hz, Me-29), 0.93 (3H, d, *J*=6.45 Hz, Me-27), 1.02 (3H, d, *J*=6.67 Hz, Me-21), 3.55 (1H, m, *J*=5.23 Hz, H-3), 5.00 (dd, *J*=15.07, 8.79 Hz), 5.12 (1H, dd, *J*=15.07, 8.79Hz), 5.33 (1H, s br, H-6);
^{13}C-NMR (126 MHz, $CDCl_3$, δ ppm), see Table 4.4;
HRMS (EI) *m/z* calcd.: 412.37052 $[M^+]$, found. 412.37047 $[M^+]$;
MS (CI, NH_3) *m/z* 413 $[MH]^+$, 397, 395 $[M-OH]^+$, 383, 271, 255

Sitosterol 3-O-β-D-galactopyranoside (**ME8**) (**92**), $C_{35}H_{60}O_6$;
white powder, **mp** 261-263 °C;
IR $\tilde{\nu}_{max}$ (KBr) cm^{-1}: 3400, 3351, 3000, 2853, 1645, 1375, 1350, 1168, 1070, 1030;
^{1}H NMR (500 MHz, DMSO-D_6, δ ppm): aglycone: 0.64 (3H, s, Me-18), 0.80 (6H, d, *J*=6.9 Hz, Me-26 and Me-27), 0.81 (3H, t, *J*=6.5 Hz, Me-29), 0.89 (3H, d, *J*=6.5 Hz, Me-21), 0.95 (3H, s, Me-19), 3.46 (1H, m, H-3), 5.31 (1H, s br, H-6); sugar: 2.88 (1H, m, H-2'), 3.00 (1H, m, H-4'), 3.1 (1H, m, H-5'), 3.30 (1H, m, H-3'), 3.40 (1H, m, H-6'), 3.62 (1H, m, H-5'), 4.20 (1H, d, *J*=7.5 Hz, H-1').
^{13}C-NMR (126 MHz, DMSO-D_6, δ ppm), see Table 4.4;
MS (ESI): *m/z* 599 $[M+Na]^+$;
MS (EI): *m/z* (rel. int. %) 414 (30) $[M_{agl.}]^+$, 396 (100), 381 (19), 329 (12). 303 (17), 273 (10), 255 (25), 231 (9), 213 (21), 175 (10), 163 (14), 161 (24), 147 (30), 145 (35), 119 (27). 107 (40), 95 (46), 81 (51).

1-(24-ferulyloxytetracosanoyl)-glycerol (**ME9**) (**93**), $C_{37}H_{62}O_8$;

brown powder; **mp** 74-76 °C.

IR $\tilde{\nu}_{max}$ (KBr) cm^{-1}: 3422 cm^{-1} (OH), 2917, 2849, 1737, 1712, 1632, 1596, 1518, 1469, 1273, 1174, 719;

^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 1.22-1.27 [36H, s, $(CH_2)18$], 1.37 (2H, m, H-21''), 1.6 (2H, m, *J*=7.2 Hz, H-22''), 1.7 (2H, t, *J*=6.8 Hz, H-2''), 2.33 (2H, t, *J*=7.5 Hz, CH_2-CO), 3.47 (1H, br, exchangeable D_2O, OH), 3.58 (1H, dd, *J*=11.3, 6.0 Hz, $\underline{CH_2}$-OH), 3.69 (1H, dd, *J*=11.5, 3.9 Hz, $\underline{CH_2}$-OH), 3.91 (3H, s, 3-OMe), 3.91 (1H, m, $\underline{CH}$OH), 4.15 (2H, d, *J*=6.0 Hz, $COOCH_2$), 4.18 (2H, t, *J*=6.8 Hz, O=C-O$\underline{CH_2}CH_2$), 6.29 (1H, d, *J*=15.9 Hz, =CH, H-2'), 6.90 (1H, d, *J*=8.2 Hz, H-5), 7.03 (1H, d, J=1.9 Hz, H-2), 7.06 (1H, dd, *J*=8.2, 1.9 Hz, H-6), 7.6 (1H, d, *J*=15.9 Hz, =CH, H-1');

HRMS (ESI-FT-ICR) *m/z* calcd.: 635.45175 $[M+H]^+$, 657.43369 $[M+Na]^+$, found: 635.45231 $[M+H]^+$, 657.43432 $[M+Na]^+$;

MS (ESI) *m/z* 657.5 $[M+Na]^+$; (ESI, negative mode) *m/z* 634.4 $[M]^+$, *m/z* 559.4 $[M-C_3H_7O_2]^+$, 541.4, 473.4, 177;

MS (CI, NH_3) *m/z* 543 $[M-C_3H_7O_3]^+$, 542, 498, 298, 296, 242 and 177;

MS (EI) *m/z* (rel. int. %) 542 (13) $[M-C_3H_8O_3]^+$, 514 (17), 498 (8), 298 (57), 296(12), 242 (23), 226 (10), 199 (17), 194 (18), 191(13), 177 (36), 97(15), 95 (17), 83 (22), 69 (35), 61 (100), 55 (54);

^{13}C-NMR (126 MHz, $CDCl_3$, δ ppm): 24.90 (C-22''), 26.00 (C-21''), 28.77 (C-2''), 29.14-29.69 (C-3''- C-20''), 34.16 (C-23''), 55.92 (OMe), 63.31 (C-3'''), 64.66 (C-1''), 65.11 (C-1'''), 70.22 (C-2'''), 109.38 (C-2), 114.83 (C-5), 115.55 (C-2'), 123.07 (C-6), 126.94 (C-1), 144.72 (C-1'), 146.87 (C-3), 148.06 (C-4), 167.50 (C-3'), 174.39 (C-24'').

Islandicin (**ME11**) (**9**), $C_{15}H_{10}O_5$;

red-brown powder, **mp** 215-217 °C;

IR $\tilde{\nu}_{max}$ (KBr) cm^{-1}: 3420, 2926, 2854, 1734, 1601, 1458, 1248, 1176, 1077, 814, 710;

^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 13.48 (1H, s, 4-OH), 12.37 (1H, s, 1-OH), 12.32 (1H, s, 8-OH), 7.88 (1H, d, *J*=7.6 Hz, H-5), 7.68 (1H, t, *J*=7.6 Hz, H-6), 7.29 (1H, d, *J*=7.6 Hz, H-7), 7.15 (1H, s, H-2), 2.37 (3H, s, Me);

^{13}C-NMR (126 MHz, $CDCl_3$, δ ppm), see Table 4.3;

HRMS (EI) *m/z* found: 270.05322 $[M^+]$, calcd.: 270.05282 $[M^+]$;

MS (EI) *m/z* (%) 270 (66) $[M]^+$, 155 (13), 141 (14), 127 (18). 99 (27), 85 (54), 69 (44), 57 (100);

β-Sitosterol (**ME12**) (**24**), $C_{29}H_{50}O$;
colorless powder, **mp** 140-141 °C;
IR $\tilde{\nu}_{max}$ (KBr) cm^{-1}: 3420, 3410, 3050, 1670, 1374, 1364, 950, 880;
^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 0.53 (3H, s, Me-18), 0.71 (3H, s, Me-19), 0.82 (3H, d, *J*=6.8 Hz, Me-26), 0.84 (3H, t, *J*=6.9 Hz, Me-29), 0.95 (3H, d, *J*=6.5 Hz, Me-27), 1.00 (3H, d, *J*=7.2 Hz, Me-21), 3.40 (1H, m, H-3), 5.30 (1H, s br, H-6);
^{13}C-NMR (126 MHz, $CDCl_3$, δ ppm), see Table 4.4;
MS (EI): *m/z* (rel. int. %) 414 (42) $[M]^+$, 396 (64), 381 (22), 273 (12), 255 (35), 231 (10), 213 (26), 175 (8), 163 (15), 161 (23), 147 (36), 145 (49), 119 (28). 109 (26), 95 (46), 81 (61).

Sitosteryl β-D-glucopyranoside (**ME13**) (**25**), $C_{35}H_{60}O_6$;
colorless powder, **mp** 258-260 °C;
IR $\tilde{\nu}_{max}$ (KBr) cm^{-1}: 3398 (OH) 2933, 2868, 1640, 1464, 1378, 1163, 1105, 1075, 1024;
^{1}H NMR (500 MHz, C_5D_5N, δ ppm): aglycone: 0.67 (3H, s, Me-18), 0.89 (6H, d, *J*=6.8 Hz, Me-26 and Me-27), 0.86 (3H, t, *J*=6.5 Hz, Me-29), 1.00 (3H, d, *J*=6.4 Hz, Me-21), 0.90 (3H, s, Me-19), 0.95 (3H, s, Me-19), 3.46 (1H, m, H-3), 5.31 (1H, s br, H-6); sugar: 2.88 (1H, m, H-2'), 3.00 (1H, m, H-4'), 3.1 (1H, m, H-5'), 3.30 (1H, m, H-3'), 3.40 (1H, m, H-6'), 3.62 (1H, m, H-5'), 4.20 (1H, d, *J*=7.8 Hz, H-1');
^{13}C-NMR (126 MHz, C_5D_5N, δ ppm), see Table 4.4;
MS (ESI): *m/z* 599 $[M+Na]^+$;
MS (EI) *m/z* (rel. int. %) 414 (24) $[M_{agl.}]^+$, 396 (100), 381 (17), 329 (11). 303 (13), 273 (8), 255 (18), 231 (7), 213 (15), 163 (14), 163 (11), 161 (19), 147 (23), 145 (23), 119 (16). 107 (24), 95 (28), 81 (23).

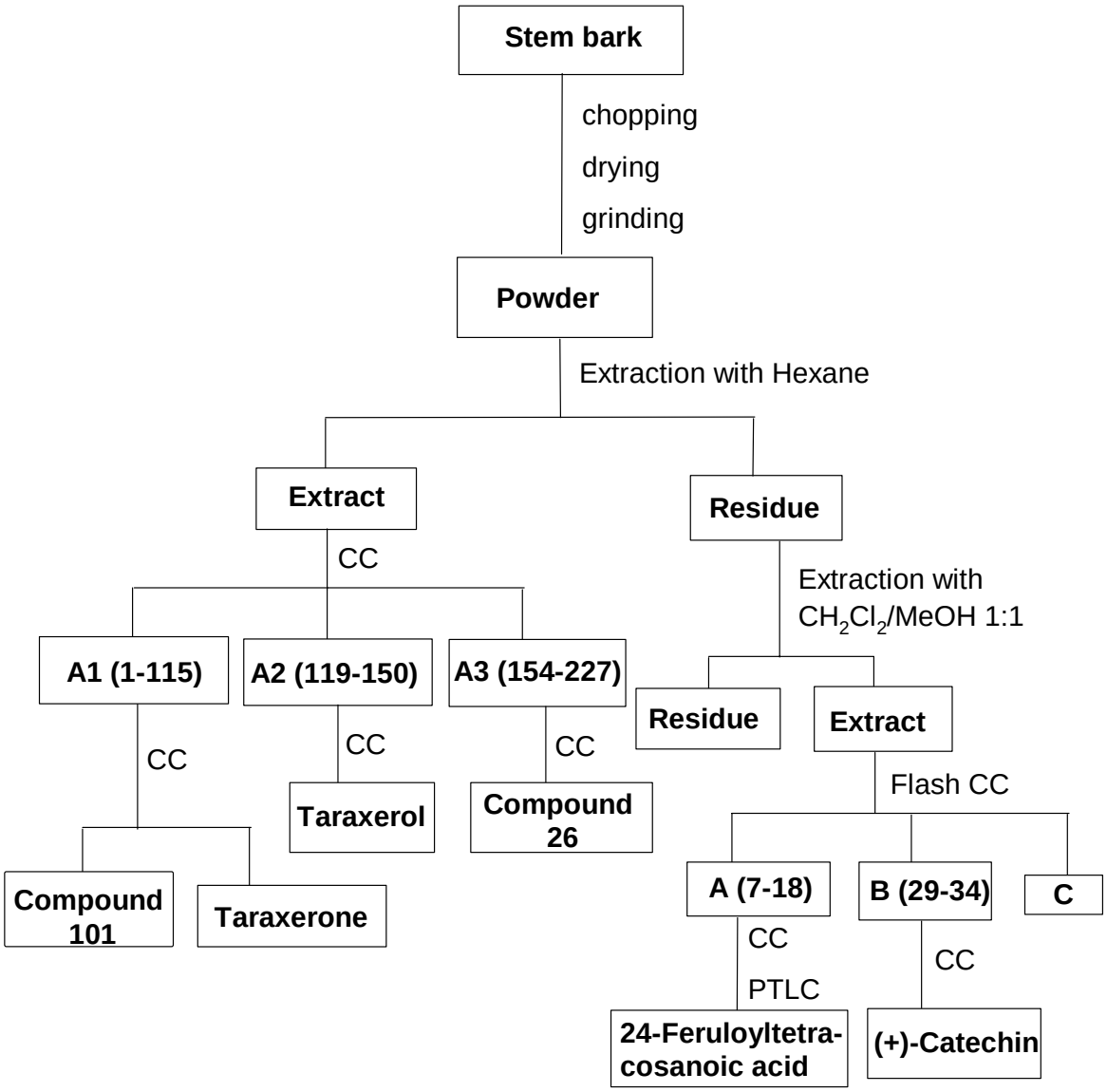
Catechin (**ME14**) (**97**+**98**), $(C_{15}H_{14}O_6)_2$,
brown powder, **mp** 87-90 °C;
IR $\tilde{\nu}_{max}$ (KBr) cm^{-1}: 3570, 3160, 1625, 1520, 1469, 1441, 1385, 1183, 1140;
^{1}H NMR and **^{13}C NMR** (see Table 4.5);
ESI-FT-ICR MS (*m/z* 603.14855; calcd for $(C_{15}H_{14}O_6)_2$+Na, 603.14730);

ESI (positive mode): *m/z* 313.1 $[M+Na]^+$ and 602.8 (base peak) $[2M+Na]^+$

6.1.2.2 Autranella congolensis

6.1.2.2.1 Extraction

Plant material (stem bark) was collected at Dschang (Western province of Cameroon), cut into pieces, air-dried and pulverized. The resulting powder has been extracted with hexane and CH_2Cl_2-MeOH.



6.1.2.2.2 Separation and purification

The residue (180 g) obtained after evaporation of the CH_2Cl_2-MeOH extract, was fractionated over silica gel (0.04-0.063 mm). Elution with a petrol ether/EtOAc mixture of increasing polarity afforded 68 fractions of 250 mL each which were collected and mixed on the basis of TLC analysis (Table 6.8).

Table 6.8: Chromatographic Fractions

Fractions	Eluent
1-5	petrol ether and petrol ether/EtOAc 4:1
6-20	petrol ether/EtOAc 3:2
21-30	petrol ether/EtOAc 2:3
31-44	petrol ether/EtOAc 1:4
45-54	EtOAc
55-62	EtOAc/MeOH 19:1
63-68	EtOAc/MeOH 9:1→4:1

Fractions 7-18 were eluted with a mixture of petrol ether/EtOAc 3:2, concentrated, and subjected to repeated column chromatography over silica gel. After isocratic elution using a mixture of CH_2Cl_2/cyclohexane/MeOH 90:10:3, 70 sub-fractions of 20 mL each were collected and monitored by TLC. Sub-fractions 20-43 were concentrated and purified by PTLC using the same solvent mixture to give **AC-F7.2**.

Fractions 29-34 were eluted with mixtures of petrol ether/EtOAc 2:3 and petrol ether/EtOAc 1:4, concentrated, and subjected to repeated column chromatography over silica gel. Elution with a mixture of CH_2Cl_2/MeOH 19:1 to 9:1 to give catechin, **AC_F32**.

The hexane extract was also subjected to column chromatography over silica gel. Elution with a mixture of petrol ether/CH_2Cl_2 1:1 to CH_2Cl_2/MeOH 19:1 resulted in collection of 250 fractions of 20 mL each, which were examined by TLC. Fractions 1-115 eluted with a petrol ether/ CH_2Cl_2 mixture of increasing polarity were concentrated and subjected to repeated column chromatography over silica gel to afford **Hex_F1** and taraxerone, **AC_Hex2.A**. Fractions 119-150 were purified on column chromatography over silica gel, eluted with pure CH_2Cl_2 to afford taraxerol, **Hex_F2**. Fractions 154-227 were purified on

column chromatography over silica gel, eluted with a CH_2Cl_2/MeOH mixture of increasing polarity to afford (24*R*)-stigmast-7,22(*E*)-dien-3α-ol, **Hex_C.1**.

Alkaline hydrolysis of Hex_F1

30 mg of **Hex_F1** was refluxed in 5% KOH solution in MeOH (5mL) and 20 mL of toluene for 20 hours. The reaction was controlled by TLC. After evaporation under reduced pressure, water was added to the mixture, followed by extraction with $CHCl_3$ to isolate the hydrolysis product, which was confirmed to be taraxerol by TLC (R_f 0.34, CH_2Cl_2/PE 1:1), MS and ^{1}H NMR. The basic solution was acidified (pH 5-7) with 10% HCl and extracted with EtOAc to isolate the mixture of fatty acids. This mixture was not further analysed.

24-Feruloyltetracosanoic acid (**AC_F7.2**) (**102**), $C_{34}H_{56}O_6$;
brown powder; **mp** 82-85 °C;
IR $\tilde{\nu}_{max}$ (KBr) cm^{-1}: 3422 (OH), 2917, 2849, 1737, 1712, 1632, 1596, 1518, 1469, 1273, 1174 and 719;
^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 1.25 [36H, s br, $(CH_2)18$], 1.37 (2H, m, H-21''), 1.61 (2H, m, *J*=7.22 Hz, H-22''), 1.7 (2H, t, *J*=6.8 Hz, H-2''), 2.34 (2H, t, *J*=7.5 Hz, $\underline{CH_2}$-CO), 5.82 (1H, br, exchangeable with D_2O, OH), 3.93 (3H, s, 3-OMe), 4.18 (2H, t, *J*=6.9 Hz, O=C-O$\underline{CH_2}CH_2$), 6.29 (1H, d, *J*=15.7 Hz, =CH, H-2'), 6.91 (1H, d, *J*=8.2 Hz, H-5), 7.03 (1H, d, *J*=1.9 Hz, H-2), 7.07 (1H, dd, *J*=8.2, 1.9 Hz, H-6), 7.61 (1H, d, *J*=15.7 Hz, =CH, H-1');
MS (EI) *m/z* (rel. int. %) 560 (14) $[M]^+$, 532 (11), 514 (20), 486 (13). 396 (24), 368 (14), 194 (47), 177 (95), 137 (26), 97 (34), 83 (47), 69 (66);
MS (ESI) *m/z* 561 $[M+H]^+$, ESI (negative mode) *m/z* 559 $[M-H]^-$;
^{13}C-NMR (125 MHz, $CDCl_3$, δ ppm): 24.69 (C-22''), 26.00 (C-21''), 28.76 (C-2''), 29.06-29.70 (C-3''- C-20''), 33.84 (C-23''),31.93 (C-), 55.93 (OMe), 64.65 (C-1''), 109.38 (C-2), 114.68 (C-5), 115.65 (C-2'), 123.06 (C-6), 127.04 (C-1), 144.66 (C-1'), 146.72 (C-3), 147.86 (C-4), 167.45 (C-3'), 178.80 (C-24'').

(24*R*)-Stigmast-7,22(*E*)-dien-3-α-ol (**Hex_C.1**) (**26**), $C_{29}H_{48}O$;
colorless powder, **mp** 156-158 °C;
IR $\tilde{\nu}_{max}$ (KBr) cm^{-1}: 3420, 2955, 2869, 1652, 1457, 1375, 1042, 971, 668;

^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 0.53 (3H, s, Me-18), 0.79 (3H, d, *J*=6.5 Hz, Me-26), 0.80 (3H, s, Me-19), 0.83 (3H, t, *J*=6.5 Hz, Me-29), 0.93 (3H, d, *J*=6.5 Hz, Me-27), 1.02 (3H, d, *J*=6.7 Hz, Me-21), 3.59 (1H, m, H-3), 5.03 (1H, ddd, *J*=16.3, 10.8, 7.6 Hz, H-23), 5.15 (1H, dd, *J*=16.3, 10.8Hz, H-22), 5.13 (1H, s, H-7).

^{13}C-NMR (126 MHz, $CDCl_3$, δ ppm), see Table 4.4;

HRMS (EI) *m/z* calcd.: 412.37047 [M^+], found: 412.37052 [M^+];

MS (EI) *m/z* (rel. int. %) 412 (30) [M]$^+$, 394 (36), 381 (31), 379 (18). 273 (19), 271 (47), 255 (100), 253(65), 229 (40), 213 (53), 173 (22), 161 (37), 145 (44), 131 (34), 119 (45). 105 (80), 81 (94), 69 (61).

(+)-Catechin (**AC_F32**) (**97**), $C_{15}H_{14}O_6$;

brown powder, **mp** 237-239 °C;

IR $\tilde{\nu}_{max}$ (KBr) cm^{-1}: 3503, 3455, 3164, 2931, 1625, 1520, 1469, 1441, 1389, 1183, 1142, 1094, 1044, 1015, 793, 626;

^{1}H NMR (500 MHz, CD_3OD, δ ppm): 2.73 (1H, dd, *J*=16.8, 2.7 Hz, H-4b), 2.85 (1H, dd, *J*=16.8, 4.6 Hz, H-4a), 4.17 (1H, m, H-3), 4.80 (1H, d, *J*=6.5 Hz, H-2), 5.91 (1H, d, *J*=2.0 Hz, H-8), δ 5.94 (1H, d, *J*=2.0 Hz, H-6), 6.75 (1H, d, *J*=8.2 Hz, H-5'), 6.79 (1H, dd, *J*=8.2, 1.9 Hz, H-6'), 6.96 (1H, d, *J*=1.9 Hz, H-2');

^{13}C-NMR (126 MHz, CD_3OD, δ ppm), see Table 4.5;

HRMS (EI) *m/z*. calcd.: 290.07815[M^+], found: 290.07904 [M^+];

MS (EI) *m/z* (rel. int. %) 290 (33) [M]$^+$, 272 (18), 163 (7), 152 (43). 139 (100), 123 (40), 77 (8), 69 (10).

Taraxerol 3-hexacosanoate, **Taraxerol 3-tetracosanoate**, **Taraxerol 3-docosanoate** (**Hex_F1**) (**101**), $C_{56}H_{100}O_2$, $C_{54}H_{96}O_2$ and $C_{52}H_{92}O_2$;

colorless powder; **mp** 176-178 °C;

IR $\tilde{\nu}_{max}$ (KBr) cm^{-1}: 2916, 2850, 1731, 1558, 1540, 1507, 1472, 1376, 1000, 668;

^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 0.81 (3H, s, Me-28), 0.85 (3H, s, Me-23), 0.87 (3H, s, Me-29), 0.88 [3H, t, *J*=5.9 Hz, Me-24'(26')(28')], 0.90 (6H, s, Me-24, Me-30), 0.95 (6H, s, Me-25, Me-26), 1.09 (3H, s, Me-27), 1.24-1.26 [36H, s br, (CH_2)18], 4.46 (1H, dd, *J*=10.4, 5.3 Hz, H-3), 5.53 (dd, *J*=8.2, 3.1 Hz, H-15);

MS (ESI-FT-ICR): *m/z* calcd.: *m/z* 805.77961 [M1+H]$^+$, calcd. for $C_{56}H_{101}O_2$, *m/z* found: 805.78011 [M1+H]$^+$; *m/z* calcd.: 777.74831 [M2+H]$^+$, calcd. for $C_{54}H_{97}O_2$, *m/z* found:

777.74865 $[M2+H]^+$ and *m/z* calcd.: 749.71701 $[M3+H]^+$, calcd. for $C_{52}H_{93}O_2$, *m/z* found: 749.71776 $[M3+H]^+$;

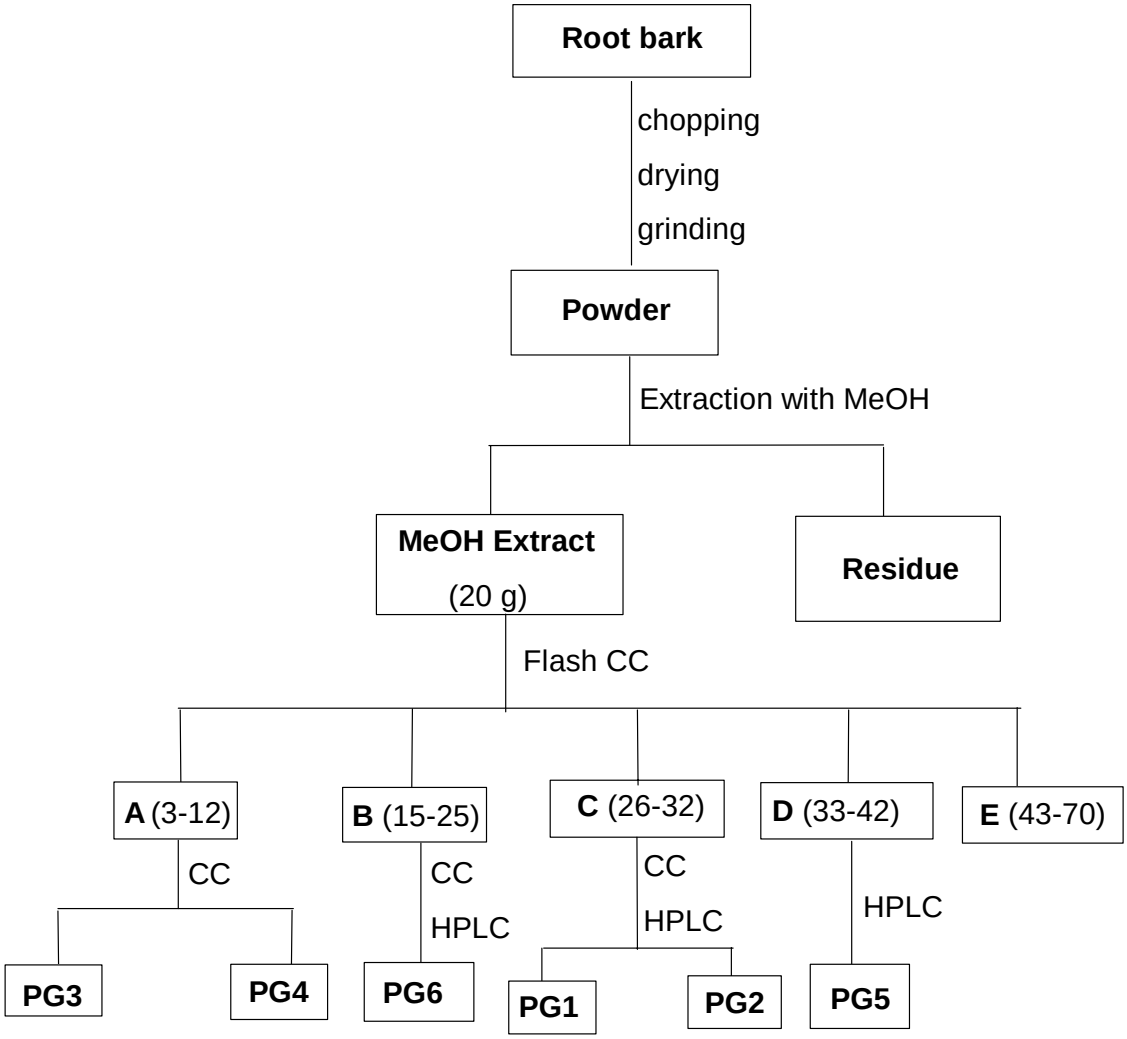
Taraxerol (**Hex_F2**) (**99**), $C_{30}H_{50}O$;
colorless powder; **mp** 276-278 °C;
IR $\tilde{\nu}_{max}$ (KBr) cm^{-1}: 3482, 2997, 2933, 2867, 1473, 1457, 1384, 815, 690, 668;
^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 0.91 (3H, s, Me-26), 0.97 (3H, s, Me-30), 1.00 (6H, s, Me-24, Me-27), 1.02 (3H, s, Me-29), 1.09 (3H, s, Me-25), 1.13 (3H, s, Me-28), 1.25 (3H, s, Me-23), 3.45 (1H, m, *J*=5.3 Hz, H-3), 5.63 (dd, *J*=8.2, 3.1 Hz, H-15), 5.80 (1H, d, *J*=5.7 Hz, 3-OH);
HRMS (EI) *m/z* calcd.: 426.38609$[M^+]$, found: 426.38617 $[M^+]$;
MS (EI) *m/z* (rel. int. %) 426 (17) $[M]^+$, 411 (10), 408 (17), 393 (21). 365 (14), 302 (35), 287 (24), 284 (21), 269 (32), 257 (13), 241 (14). 218 (28), 204 (100), 203 (17), 189 (34), 121 (36), 109 (28). 105 (28), 95 (33), 81 (28).

Taraxerone (**AC_Hex2.A**) (**100**), $C_{30}H_{48}O$;
white powder; **mp** 240-242 °C;
IR $\tilde{\nu}_{max}$ (KBr) cm^{-1}: 3047, 2939, 2863, 1708, 1473, 1376, 995, 816, 687;
^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 0.80 (3H, s, Me-26), 0.88 (3H, s, Me-28), 0.89 (3H, s, Me-24), 0.93 (3H, s, Me-29), 1.04 (3H, s, Me-30), 1.05 (3H, s, Me-25), 1.06 (3H, s, Me-23), 1.11 (3H, s, Me-27), 5.53 (dd, J=8.2, 3.1 Hz, H-15).
MS (ESI-FT-ICR): *m/z* calcld.: 425.37747 $[M+H]^+$, 447.35936 $[M+Na]^+$, found: 425.37779 $[M+H]^+$, 447.35974 $[M+Na]^+$;
MS (ESI): 425 $[M+H]^+$, 849 $[2M+H]^+$.

6.1.2.3 Pentadesma grandifolia

6.1.2.3.1 Extraction

Plant material (root bark) was collected at Bagangté (Western Province of Cameroon in December 2001), cut into pieces, air-dried and pulverized. Voucher specimen documenting the collection are deposited at the National Herbarium, Yaoundé-Cameroon. The resulting powder has been extracted with MeOH.



6.1.2.3.2 Separation and purification

A part of residue (20 g) obtained after evaporation of the MeOH extract, was fractionated over silica gel. Elution with a petrol ether/EtOAc mixture of increasing polarity, followed by EtOAc and a mixture of EtOAc/MeOH 9:1 to 4:1 respectively, afforded 70 fractions of 250 mL each which were collected and combined on the basis of TLC analysis (see chromatogram below, Table 6.9)

Table 6.9: Chromatographic Fractions of the MeOH Extract

Fractions	Eluent
1-15	petrol ether and petrol ether/EtOAc 9:1
16-27	petrol ether/EtOAc 4:1
28-40	petrol ether/EtOAc 3:2
41-49	petrol ether/EtOAc 2 :3
50-60	EtOAc
61-70	EtOAc/MeOH 9:1→4:1

Fractions 3-12 (oily mixture) were eluted with petrol ether and a mixture of petrol ether/EtOAc 9:1, concentrated, and subjected to repeated column chromatography over silica gel, eluted with a mixture of petrol ether/EtOAc 19:1 and petrol ether/EtOAc 23:2. Sub-fractions 14-27 were eluted with petrol ether/EtOAc 19:1 to give **PG4** (1 g) as a viscous oil. **PG3** (150 mg) precipitated from the sub-fractions 49-90, eluted with a mixture of petrol ether/EtOAc 23:2.

Fractions 15-25 eluted with a mixture of petrol ether/EtOAc 9:1 and petrol ether/EtOAc 4:1 was chromatographed a second time over silica gel (0.04-0.063 mm) to give a yellow powder which after further purification on HPLC, using preparative method 2, yield **PG6** (4 mg), retention time 41.8 min.

Fractions 26-32 eluted with the mixture of petrol ether/EtOAc 4:1 and petrol ether/EtOAc 3:2 was chromatographed a second time over silica gel. Isocratic elution using a mixture of $CHCl_3$-MeOH 100:1 results in 110 fractions (ca 5 mL per fraction). The sub-fractions 24-52 crystallised to give a yellow powder (700 mg), which was further purified on preparative HPLC to give **PG1** as a yellow gum (200 mg), having a retention time 30.3 min. The subfractions 53-68 crystallised to give **PG2** as a yellowish powder (130 mg).

Fractions 33-42 were evaporated and subjected to preparative HPLC using method 1 to yield **PG5** (3.5 mg) having the retention time 36.7 min.

α-Mangostin (**PG1**) (**103**), $C_{24}H_{26}O_6$;

yellow gum (200 mg);

IR $\tilde{\nu}_{max}$ (KBr) cm^{-1}: 3405, 3101, 2978, 2934, 1779, 1605, 1463, 1375, 1284, 1158, 816, 719;

^{1}H NMR (500 MHz, CD_3OD, δ ppm): 6.63 (1H, s, H-5), 6.18 (1H, s, H-4), 5.21 (2H, m, H-2' and H-2''), 4.87 (3H, s, 1-OH, 3-OH, 6-OH), 4.02 (2H, d, *J* = 6.3 Hz, H-1''), 3.73 (3H, s, 7-OMe), 3.24 (2H, d, *J*=7.5 Hz, H-1'), 1.80 (3H, s br, H_3-5''), 1.76 (3H, s, H_3-5'), 1.65 (3H, s, H_3-4''), 1.64 (3H, s, H_3-4');

HRMS (EI) *m/z* calcd.: 410.17294 $[M]^+$; found: 410.17240 $[M]^+$;

MS (EI) *m/z* (rel. int. %) 410 (67) $[M]^+$, 367 (42), 355 (34), 354 (38), 353 (20), 340 (21), 339 (100), 323(21), 321 (17), 311 (23), 297 (12), 285 (14), 162 (10), 115 (7), 77(10), 69 (9).

Rubraxanthone (**PG2**) (**104**), $C_{24}H_{26}O_6$;

yellowish powder (130 mg), **mp** 205-207 °C;

IR $\tilde{\nu}_{max}$ (KBr) cm^{-1}: 3432, 2968, 2914, 2838, 1646, 1607, 1577, 1513, 1467, 1433, 1298, 1270, 1163, 1115, 1077, 1030, 841, 827, 627;

^{1}H NMR (600 MHz, CD_3OD, δ ppm): 6.68 (1H, s, H-5), 6.17 (1H, d, *J*=2.0 Hz, H-4), 6.08 (1H, d, *J*=2.0 Hz, H-2), 5.20 (1H, t, *J*=6.3 Hz, H-2'), 5.04 (1H, t, *J*=6.9 Hz, H-7'), 4.04 (2H, d, *J*=6.3 Hz, H-1'), 3.76 (3H, s, 7-OMe), 2.04 (2H, m, H-6'), 1.96 (2H, m, H-4'), 1.80 (3H, s, H_3-5'), 1.54 (3H, s, H_3-9'), 1.51 (3H, s, H_3-10');

HRMS (EI) *m/z* calcd.: 410.17294 $[M]^+$; found: 410.17366 $[M]^+$;

MS (EI) *m/z* (rel. int. %) 410 (21) $[M]^+$, 341 (100), 342 (21), 311 (15), 309 (11), 299 (23), 288 (13), 271 (7), 69 (11).

Lupeol (**PG3**) (**23**), $C_{30}H_{50}O$;

colorless powder (150 mg), **mp** 213-215 °C (Lit. Reynolds, 1986; 215-216 °C);

IR $\tilde{\nu}_{max}$ (KBr) cm^{-1}: 3318, 2931, 2870, 1734, 1673, 1638, 1486, 1452, 1379, 1257, 1241, 1042, 933, 880, 806;

^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 4.68 (1 H, d, *J*=2.0 Hz), 4.56 (1 H, d, *J*=2.0 Hz), 1.68 (3 H, s, Me-30), 1.03 (3 H, s, Me-26), 0.97 (3 H, s, Me-23), 0.94 (3 H, s, Me-27), 0.83 (3 H, s, Me-25), 0.79 (3 H, s, Me-28), 0.76 (3 H, s, Me-24), 0.68 (1 H, d, *J*=9.4 Hz, H-5);

HRMS (EI) *m/z* calcd.: 426.38617 $[M^+]$, found: 426.38650 $[M^+]$;

MS (EI) *m/z* (rel. int. %) 426 (54) $[M]^+$, 411 (17), 408 (26), 354 (38), 393 (14), 365 (22), 316 (10), 315 (13), 297 (15), 257 (13), 234 (14), 229 (21), 218 (76), 207 (57), 203(54), 189 (86), 161 (42), 147 (46), 135 (83), 121 (84), 109 (84), 95 (100), 79 (52), 55 (82).

(13*E*,17*E*)-Polypoda-7,13,17,21-tetraen-3β-ol (PG4) (105), $C_{30}H_{50}O$;

yellow oil (1g);

IR $\tilde{\nu}_{max}$ (KBr) cm^{-1}: 3376, 2926, 1443, 1383, 1091, 1060;

^{1}H NMR (500 MHz, CD_3OD, δ ppm): 5.38 (1H, S, br H-7), 5.12 (3H, m, H-13, H-17, H-21), 3.22 (1H, dd, J = 4.55, 11.14 Hz, H-3), 1.71 (3H, br s, Me-26), 1.68 (3H, br s, Me-30), 1.61 (6H, br s, Me-28 and Me-29), 1.59 (3H, s, Me-27), 0.96 (3H, br s, Me-23), 0.84 (3H, br s, Me-24), 0.74 (3H, br s, Me-25);

MS (ESI-FT-ICR) *m/z* calcd.: 427.39344 $[M+H]^+$, 444.41999 $[M+NH_4]^+$, 449.37539 $[M+Na]^+$, found: 427.39315 $[M+H]^+$, 444.41954 $[M+NH_4]^+$, 449.37499 $[M+Na]^+$;

MS (EI) *m/z* (rel. int. %) 426 (5) $[M]^+$, 408, 339 (4), 271 (5), 203 (10), 189 (16), 187 (31), 175 (10), 147 (15), 137 (16), 123 (19), 121 (22), 107 (22), 95 (26), 81 (64), 68 (14), 69 (100), 55 (26), 41 (49);

MS (CI, NH_3) *m/z* (rel. int. %) 444 (55) $[M+NH_4]^+$, 427 (38) $[M+H]^+$, 409 (100) $[M+H-H_2O]^+$, 203 (10), 137 (16), 95 (10);

MS (ESI) *m/z* 427 $[M+H]^+$;

MS (ESI, negative mode) *m/z* 461.8 $[M+Cl]^-$.

Garcinone E (PG5) (106), $C_{28}H_{32}O_6$;

yellow powder (3.5 mg);

^{1}H NMR (600 MHz, $(CD)_3CO$, δ ppm): 13.92 (1H, s, 1-OH), 6.47 (1H, s, H-4), 5.28 (1H, m, H-2'), 5.27 (1H, m, H-2''), 5.25 (1H, m, H-2'''), 4.20 (2H, d, *J*=6.8 Hz, H-1'''), 3.60 (2H, d, *J*=7.1 Hz, H-1''), 3.35 (2H, d, *J*=7.1 Hz, H-1'), 1.88 (3H, s, H_3-4''), 1.81 (3H, s, H_3-4'''), 1.78 (3H, s, H_3-4'), 1.65 (6H, s, H_3-5' and H_3-5''), 1.63 (6H, s, H_3-5'''');

MS (ESI-FT-ICR) *m/z* calcd.: 465.22717 $[M+H]^+$, 487.20911 $[M+Na]^+$, found: 465.22735 $[M+H]^+$, 487.20918 $[M+Na]^+$,

MS (ESI) *m/z* 465 $[M+H]^+$,

MS (ESI, negative mode) *m/z* 463 $[M-H]^-$.

Cowanin (**PG6**) (**108**), $C_{29}H_{34}O_6$;

amorphous yellow solide (4 mg);

IR $\tilde{\nu}_{max}$ (KBr) cm^{-1}: 3433, 1643, 1611, 1583, 1451, 1160;

^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 13.76 (1H, s, 1-OH), 6.81 (1H, s, H-5), 6.27 (1H, s, H-4), 5.24 (2H, m, H-2', H-2''), 5.00 (1H, m, H-7''), 4.08 (2H, d, *J*=6.1 Hz, H-1''), 3.78 (3H, s, 7-OMe), 3.44 (2H, d, *J*=7.1 Hz, H-1'), 1.99 (4H, m, H-4'', H-6''), 1.82 (3H, br s, H_3-4'), 1.80 (3H, br s, H_3-5''), 1.74 (3H, s, H_3-5'), 1.58 (3H, s, H_3-9''), 1.52 (3H, s, H_3-10'');

HRMS (EI) *m/z* calcd.: 478.23554 [M^+], found: 478.23545 [M^+];

MS (EI) *m/z* (rel. int. %) 478 (47) $[M]^+$, 423 (10), 409 (100), 367 (34), 356 (57), 353 (66), 339 (16), 313 (14), 301 (28), 299 (15), 297 (13), 285 (11), 257 (6), 81 (8), 69 (15).

7 Annex

7.1 Selected HMBC correlations of compound ME1

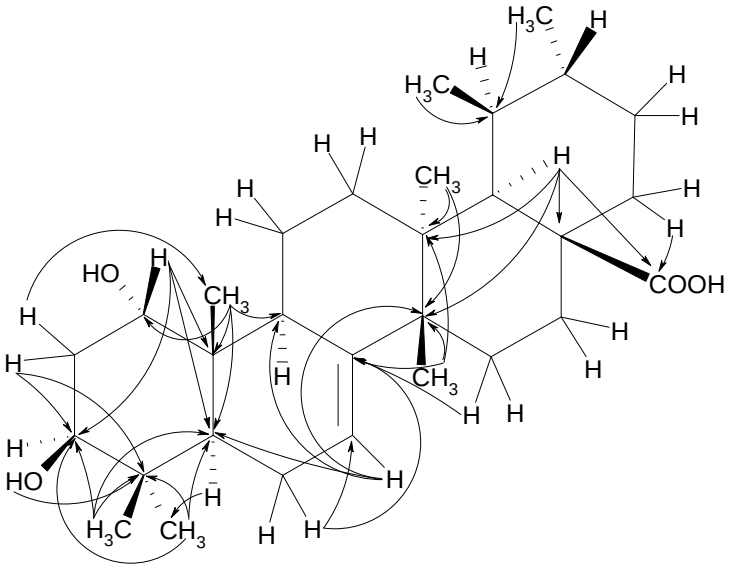


Figure7.1

7.2 Information on the X-ray analysis

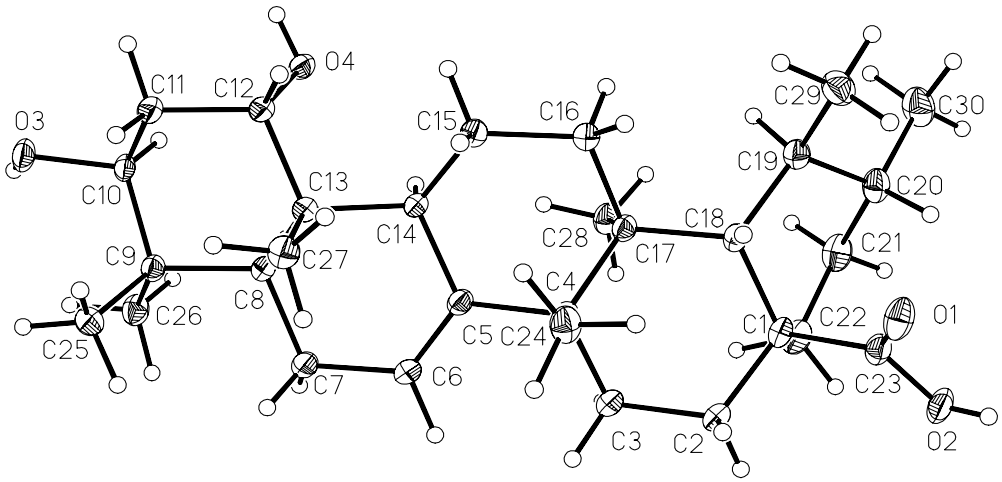


Figure 7.2: Molecular structure (relative configuration) of compound **85** according to X-ray analysis

Table 7.1. Crystal data and structure refinement for compound 1.

Measurement device	Nonius KappaCCD
Empirical formula	C_{30} H_{48} O_4
Formula weight	472.68
Temperature	100(2) K
Wavelength	0.71073 A
Crystal system, space group	Orthorhombic P 2_1 2_1 2_1
Unit cell dimensions	a = 7.36000(10) A. b = 12.31600(10) A. c = 29.2710(4) A.
Volume	2653.29(6) A^3
Z, Calculated density	4, 1.183 Mg/m^3
Absorption coefficient	0.076 mm^{-1}
F(000)	1040
Crystal size, colour and habit	0.30 x 0.30 x 0.08 mm^3,Colourless plate
Theta range for data collection	3.10 to 30.00 deg.
Index ranges	-10<=h<=10, -17<=k<=17, -40<=l<=41
Reflections collected / unique	30886 / 7687 [R(int) = 0.037]
Completeness to theta = 30.00	99.6%
Absorption correction	multi-scan
Max. and min. transmission	0.9939 and 0.9775
Programs used for solution and refinement	SHELX-97
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	7687 / 0 / 499
Goodness-of-fit on F^2	1.039
Final R indices [I>2sigma(I)]	R1 = 0.0339, wR2 = 0.0837 [7094]
R indices (all data)	R1 = 0.0384, wR2 = 0.0861
Absolute structure parameter	-0.2(5)
Largest diff. peak and hole	0.278 and -0.157 $e.A^{-3}$
Remarks	Hydrogens were refined isotropically.

Table 7.2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (A^2 x 10^3) for compound 1.
U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.

	x	y	z	U(eq)
O(1)	461(1)	10792(1)	5846(1)	23(1)
O(2)	3086(1)	10804(1)	5468(1)	23(1)
O(3)	3665(1)	7953(1)	9872(1)	22(1)
O(4)	1866(1)	7101(1)	8569(1)	18(1)
C(1)	3080(2)	9940(1)	6206(1)	17(1)
C(2)	3833(2)	10897(1)	6497(1)	21(1)
C(3)	4467(2)	10553(1)	6974(1)	21(1)
C(4)	2948(1)	9989(1)	7245(1)	15(1)
C(5)	3570(2)	9659(1)	7725(1)	15(1)
C(6)	4990(2)	10114(1)	7934(1)	18(1)
C(7)	5518(2)	9907(1)	8425(1)	20(1)
C(8)	4377(1)	9035(1)	8667(1)	14(1)
C(9)	4691(1)	9019(1)	9198(1)	15(1)
C(10)	3558(2)	8063(1)	9379(1)	16(1)
C(11)	1563(2)	8155(1)	9261(1)	18(1)
C(12)	1291(2)	8135(1)	8742(1)	16(1)
C(13)	2376(1)	9042(1)	8499(1)	14(1)
C(14)	2457(2)	8796(1)	7976(1)	14(1)
C(15)	594(2)	8575(1)	7758(1)	19(1)
C(16)	610(2)	8476(1)	7230(1)	19(1)
C(17)	2303(2)	8951(1)	6981(1)	15(1)
C(18)	1705(1)	9240(1)	6480(1)	15(1)
C(19)	1037(2)	8231(1)	6195(1)	17(1)
C(20)	2037(2)	8093(1)	5735(1)	21(1)
C(21)	4092(2)	8188(1)	5804(1)	24(1)
C(22)	4678(2)	9276(1)	6013(1)	23(1)
C(23)	2050(2)	10528(1)	5824(1)	18(1)
C(24)	1390(2)	10825(1)	7310(1)	22(1)
C(25)	4214(2)	10078(1)	9454(1)	20(1)
C(26)	6714(2)	8777(1)	9287(1)	21(1)
C(27)	1410(2)	10135(1)	8585(1)	19(1)
C(28)	3824(2)	8086(1)	6992(1)	19(1)
C(29)	-1021(2)	8267(1)	6123(1)	24(1)
C(30)	1566(2)	7008(1)	5505(1)	29(1)

Table 7.3. Bond distances in compound 1

Bond	Distance [Å]	Bond	Distance [Å]
O(1)-C(23)	1.2157(14)	C(14)-H(14)	0.972(13)
O(2)-C(23)	1.3334(13)	C(15)-C(16)	1.5493(14)
O(2)-H(2)	0.797(19)	C(15)-H(15A)	0.996(16)
O(3)-C(10)	1.4509(12)	C(15)-H(15B)	0.999(15)
O(3)-H(3)	0.80(2)	C(16)-C(17)	1.5581(15)
O(4)-C(12)	1.4354(12)	C(16)-H(16A)	0.983(18)
O(4)-H(4)	0.845(18)	C(16)-H(16B)	0.980(16)
C(1)-C(23)	1.5344(14)	C(17)-C(28)	1.5453(15)
C(1)-C(22)	1.5406(15)	C(17)-C(18)	1.5721(14)
C(1)-C(18)	1.5518(15)	C(18)-C(19)	1.5748(14)
C(1)-C(2)	1.5565(15)	C(18)-H(18)	0.992(15)
C(2)-C(3)	1.5306(15)	C(19)-C(29)	1.5302(16)
C(2)-H(2A)	0.965(17)	C(19)-C(20)	1.5447(15)
C(2)-H(2B)	1.010(17)	C(19)-H(19)	1.014(15)
C(3)-C(4)	1.5369(15)	C(20)-C(21)	1.5306(17)
C(3)-H(3A)	0.989(17)	C(20)-C(30)	1.5355(17)
C(3)-H(3B)	0.922(15)	C(20)-H(20)	1.027(16)
C(4)-C(5)	1.5326(13)	C(21)-C(22)	1.5353(18)
C(4)-C(24)	1.5520(15)	C(21)-H(21A)	0.968(16)
C(4)-C(17)	1.5690(14)	C(21)-H(21B)	0.985(17)
C(5)-C(6)	1.3340(15)	C(22)-H(22A)	0.961(15)
C(5)-C(14)	1.5289(14)	C(22)-H(22B)	1.014(16)
C(6)-C(7)	1.5101(14)	C(24)-H(24A)	0.982(17)
C(6)-H(6)	1.002(16)	C(24)-H(24B)	0.993(17)
C(7)-C(8)	1.5372(14)	C(24)-H(24C)	0.978(17)
C(7)-H(7A)	0.991(16)	C(25)-H(25A)	0.989(14)
C(7)-H(7B)	0.992(17)	C(25)-H(25B)	0.929(18)
C(8)-C(13)	1.5533(15)	C(25)-H(25C)	0.993(17)
C(8)-C(9)	1.5714(13)	C(26)-H(26A)	1.020(15)
C(8)-H(8)	0.991(14)	C(26)-H(26B)	1.000(14)
C(9)-C(10)	1.5373(14)	C(26)-H(26C)	0.993(15)
C(9)-C(26)	1.5405(15)	C(27)-H(27A)	0.955(15)
C(9)-C(25)	1.5447(14)	C(27)-H(27B)	0.996(18)
C(10)-C(11)	1.5130(16)	C(27)-H(27C)	0.945(15)
C(10)-H(10)	1.010(13)	C(28)-H(28A)	0.957(19)
C(11)-C(12)	1.5316(14)	C(28)-H(28B)	0.964(17)
C(11)-H(11A)	0.979(14)	C(28)-H(28C)	0.98(2)
C(11)-H(11B)	0.994(15)	C(29)-H(29A)	0.947(17)
C(12)-C(13)	1.5462(14)	C(29)-H(29B)	0.973(16)
C(12)-H(12)	0.980(15)	C(29)-H(29C)	1.032(17)
C(13)-C(27)	1.5431(14)	C(30)-H(30A)	0.972(18)
C(13)-C(14)	1.5617(14)	C(30)-H(30B)	0.988(17)
C(14)-C(15)	1.5362(15)	C(30)-H(30C)	1.029(17)

Table 7.4. Bond angles in compound 1

	Angle [deg]		Angle [deg]
C(23)-O(2)-H(2)	107.3(14)	C(17)-C(16)-H(16A)	108.7(10)
C(10)-O(3)-H(3)	109.1(16)	C(15)-C(16)-H(16B)	111.6(9)
C(12)-O(4)-H(4)	105.7(11)	C(17)-C(16)-H(16B)	105.7(10)
C(23)-C(1)-C(22)	111.02(8)	H(16A)-C(16)-H(16B)	103.7(14)
C(23)-C(1)-C(18)	108.45(9)	C(28)-C(17)-C(16)	108.10(9)
C(22)-C(1)-C(18)	113.16(9)	C(28)-C(17)-C(4)	109.40(8)
C(23)-C(1)-C(2)	102.62(8)	C(16)-C(17)-C(4)	108.46(8)
C(22)-C(1)-C(2)	109.34(9)	C(28)-C(17)-C(18)	112.29(8)
C(18)-C(1)-C(2)	111.75(8)	C(16)-C(17)-C(18)	107.38(9)
C(3)-C(2)-C(1)	113.45(9)	C(4)-C(17)-C(18)	111.08(8)
C(3)-C(2)-H(2A)	108.4(9)	C(1)-C(18)-C(17)	115.13(9)
C(1)-C(2)-H(2A)	106.2(9)	C(1)-C(18)-C(19)	111.64(8)
C(3)-C(2)-H(2B)	108.4(9)	C(17)-C(18)-C(19)	113.68(8)
C(1)-C(2)-H(2B)	110.2(9)	C(1)-C(18)-H(18)	104.5(8)
H(2A)-C(2)-H(2B)	110.1(13)	C(17)-C(18)-H(18)	105.1(8)
C(2)-C(3)-C(4)	111.98(10)	C(19)-C(18)-H(18)	105.6(8)
C(2)-C(3)-H(3A)	108.7(10)	C(29)-C(19)-C(20)	110.73(9)
C(4)-C(3)-H(3A)	109.6(10)	C(29)-C(19)-C(18)	111.06(9)
C(2)-C(3)-H(3B)	112.0(8)	C(20)-C(19)-C(18)	113.58(9)
C(4)-C(3)-H(3B)	110.2(8)	C(29)-C(19)-H(19)	106.7(9)
H(3A)-C(3)-H(3B)	104.0(13)	C(20)-C(19)-H(19)	105.2(8)
C(5)-C(4)-C(3)	112.09(9)	C(18)-C(19)-H(19)	109.2(8)
C(5)-C(4)-C(24)	106.54(8)	C(21)-C(20)-C(30)	110.30(10)
C(3)-C(4)-C(24)	107.51(9)	C(21)-C(20)-C(19)	110.34(9)
C(5)-C(4)-C(17)	109.03(8)	C(30)-C(20)-C(19)	111.75(10)
C(3)-C(4)-C(17)	109.50(8)	C(21)-C(20)-H(20)	110.3(9)
C(24)-C(4)-C(17)	112.18(9)	C(30)-C(20)-H(20)	107.2(9)
C(6)-C(5)-C(14)	119.44(9)	C(19)-C(20)-H(20)	106.8(9)
C(6)-C(5)-C(4)	122.89(9)	C(20)-C(21)-C(22)	113.36(10)
C(14)-C(5)-C(4)	117.64(9)	C(20)-C(21)-H(21A)	110.3(10)
C(5)-C(6)-C(7)	124.57(10)	C(22)-C(21)-H(21A)	107.7(9)
C(5)-C(6)-H(6)	120.1(9)	C(20)-C(21)-H(21B)	107.7(10)
C(7)-C(6)-H(6)	115.1(9)	C(22)-C(21)-H(21B)	111.2(10)
C(6)-C(7)-C(8)	114.57(9)	H(21A)-C(21)-H(21B)	106.4(13)
C(6)-C(7)-H(7A)	106.9(9)	C(21)-C(22)-C(1)	113.30(10)
C(8)-C(7)-H(7A)	109.6(9)	C(21)-C(22)-H(22A)	109.9(8)
C(6)-C(7)-H(7B)	109.0(8)	C(1)-C(22)-H(22A)	110.2(9)
C(8)-C(7)-H(7B)	108.1(9)	C(21)-C(22)-H(22B)	109.6(9)
H(7A)-C(7)-H(7B)	108.4(13)	C(1)-C(22)-H(22B)	109.3(9)
C(7)-C(8)-C(13)	111.59(8)	H(22A)-C(22)-H(22B)	104.2(13)
C(7)-C(8)-C(9)	112.59(8)	O(1)-C(23)-O(2)	121.59(10)
C(13)-C(8)-C(9)	116.98(8)	O(1)-C(23)-C(1)	124.23(10)
C(7)-C(8)-H(8)	106.8(8)	O(2)-C(23)-C(1)	114.01(10)
C(13)-C(8)-H(8)	104.4(8)	C(4)-C(24)-H(24A)	112.9(10)
C(9)-C(8)-H(8)	103.3(8)	C(4)-C(24)-H(24B)	110.1(10)
C(10)-C(9)-C(26)	108.56(9)	H(24A)-C(24)-H(24B)	106.3(13)

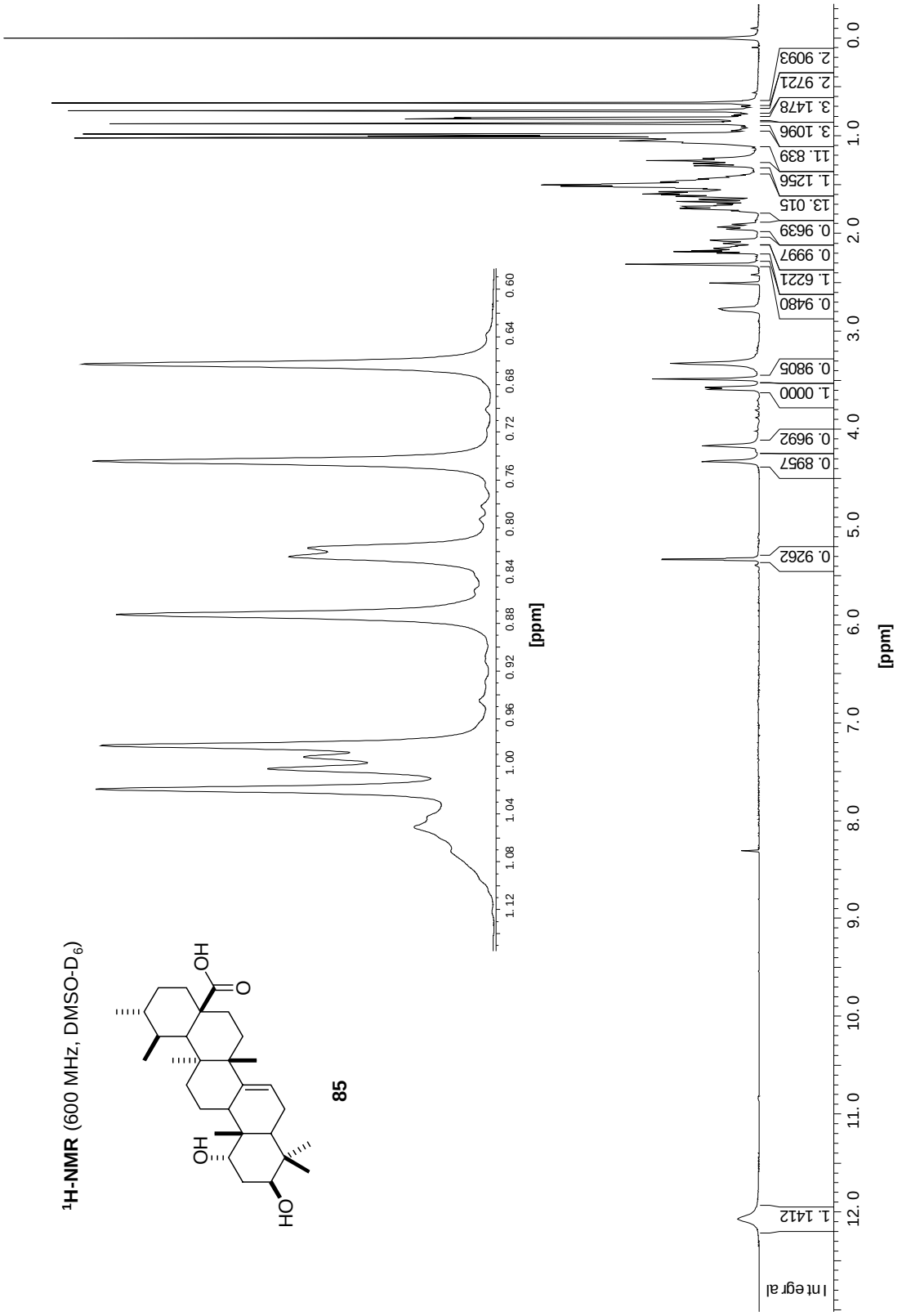
C(10)-C(9)-C(25)	110.88(8)	C(4)-C(24)-H(24C)	111.3(10)
C(26)-C(9)-C(25)	107.52(9)	H(24A)-C(24)-H(24C)	109.8(14)
C(10)-C(9)-C(8)	105.66(8)	H(24B)-C(24)-H(24C)	106.0(13)
C(26)-C(9)-C(8)	108.15(8)	C(9)-C(25)-H(25A)	108.0(9)
C(25)-C(9)-C(8)	115.87(8)	C(9)-C(25)-H(25B)	113.9(10)
O(3)-C(10)-C(11)	106.64(8)	H(25A)-C(25)-H(25B)	105.0(13)
O(3)-C(10)-C(9)	106.6(8)	C(9)-C(25)-H(25C)	110.5(9)
C(9)-C(10)-H(10)	110.0(8)	H(25A)-C(25)-H(25C)	110.8(13)
C(10)-C(11)-C(12)	110.58(8)	H(25B)-C(25)-H(25C)	108.3(14)
C(10)-C(11)-H(11A)	109.7(9)	C(9)-C(26)-H(26A)	110.4(9)
C(12)-C(11)-H(11A)	110.4(8)	C(9)-C(26)-H(26B)	109.8(9)
C(10)-C(11)-H(11B)	110.4(9)	H(26A)-C(26)-H(26B)	108.7(12)
C(12)-C(11)-H(11B)	107.9(8)	C(9)-C(26)-H(26C)	110.9(8)
H(11A)-C(11)-H(11B)	107.8(12)	H(26A)-C(26)-H(26C)	109.1(12)
O(4)-C(12)-C(11)	109.06(8)	H(26B)-C(26)-H(26C)	108.0(12)
O(4)-C(12)-C(13)	108.99(8)	C(13)-C(27)-H(27A)	113.1(9)
C(11)-C(12)-C(13)	112.22(8)	C(13)-C(27)-H(27B)	110.4(10)
O(4)-C(12)-H(12)	111.0(8)	H(27A)-C(27)-H(27B)	105.0(13)
C(11)-C(12)-H(12)	108.8(8)	C(13)-C(27)-H(27C)	111.5(9)
C(13)-C(12)-H(12)	106.7(8)	H(27A)-C(27)-H(27C)	109.9(13)
C(27)-C(13)-C(12)	108.50(8)	H(27B)-C(27)-H(27C)	106.5(13)
C(27)-C(13)-C(8)	112.95(8)	C(17)-C(28)-H(28A)	109.8(11)
C(12)-C(13)-C(8)	109.81(8)	C(17)-C(28)-H(28B)	110.0(10)
C(27)-C(13)-C(14)	110.23(8)	H(28A)-C(28)-H(28B)	110.3(15)
C(12)-C(13)-C(14)	109.40(8)	C(17)-C(28)-H(28C)	112.5(11)
C(8)-C(13)-C(14)	105.89(8)	H(28A)-C(28)-H(28C)	110.0(16)
C(5)-C(14)-C(15)	113.74(8)	H(28B)-C(28)-H(28C)	104.0(15)
C(5)-C(14)-C(13)	110.88(8)	C(19)-C(29)-H(29A)	114.3(10)
C(15)-C(14)-C(13)	114.00(8)	C(19)-C(29)-H(29B)	109.2(10)
C(5)-C(14)-H(14)	106.4(8)	H(29A)-C(29)-H(29B)	105.2(13)
C(15)-C(14)-H(14)	108.8(8)	C(19)-C(29)-H(29C)	110.0(10)
C(13)-C(14)-H(14)	102.1(8)	H(29A)-C(29)-H(29C)	107.5(14)
C(14)-C(15)-C(16)	114.88(9)	H(29B)-C(29)-H(29C)	110.4(13)
C(14)-C(15)-H(15A)	108.4(9)	C(20)-C(30)-H(30A)	110.8(10)
C(16)-C(15)-H(15A)	108.5(9)	C(20)-C(30)-H(30B)	110.6(9)
C(14)-C(15)-H(15B)	107.9(9)	H(30A)-C(30)-H(30B)	110.4(14)
C(16)-C(15)-H(15B)	110.4(9)	C(20)-C(30)-H(30C)	110.8(9)
H(15A)-C(15)-H(15B)	106.3(13)	H(30A)-C(30)-H(30C)	104.9(14)
C(15)-C(16)-C(17)	116.34(9)	H(30B)-C(30)-H(30C)	109.3(13)
C(15)-C(16)-H(16A)	109.9(9)		

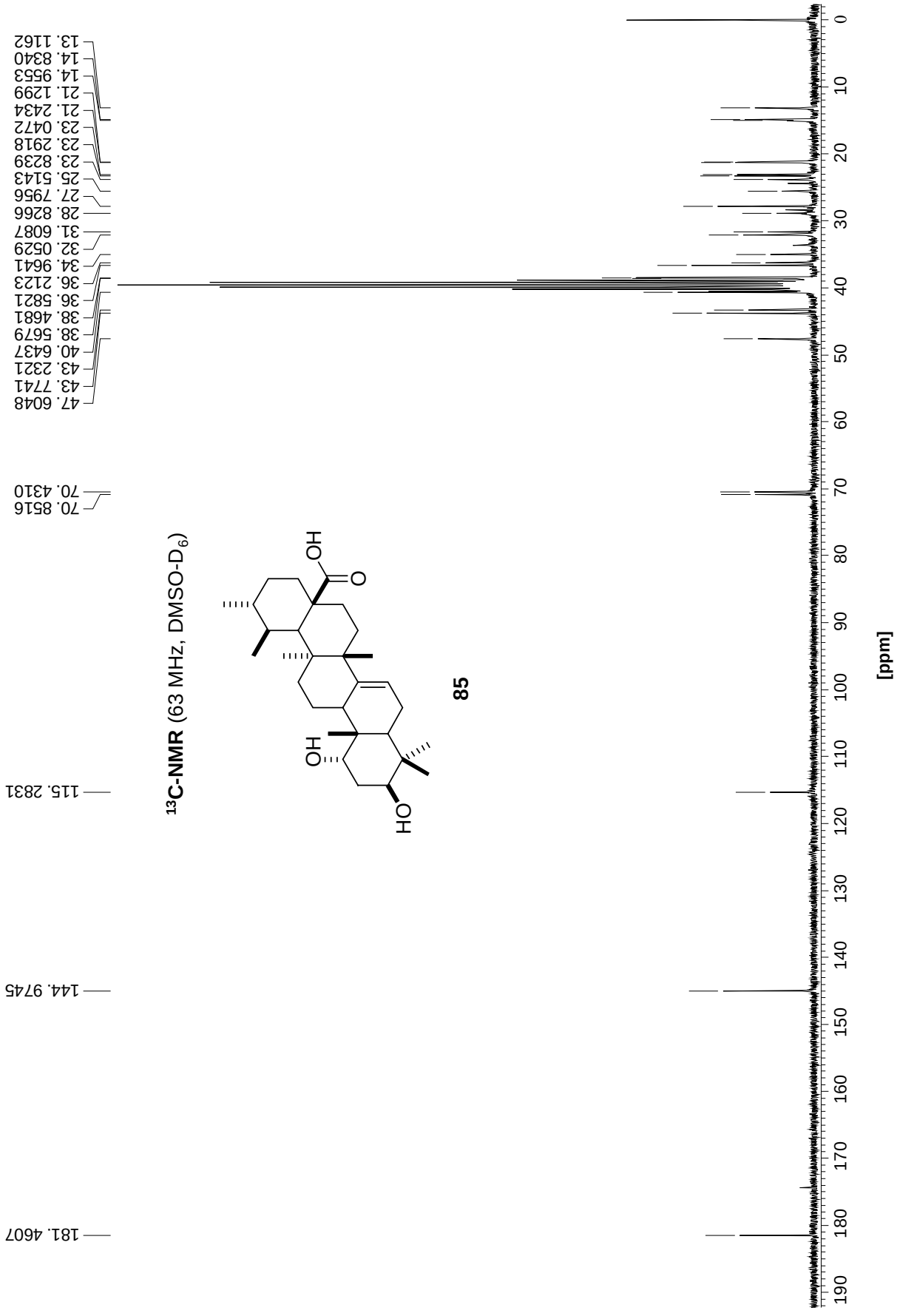
Table 7.5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters (A^2 x 10^3) for compound 1

	x	y	z	U(eq)
H(2)	2480(3)	11158(15)	5300(6)	41(5)
H(3)	4640(3)	7720(18)	9939(7)	56(6)
H(4)	1250(2)	6634(13)	8713(6)	31(4)
H(2A)	4870(2)	11179(12)	6333(5)	26(4)
H(2B)	2880(2)	11480(13)	6532(5)	29(4)
H(3A)	4880(2)	11205(14)	7141(6)	34(4)
H(3B)	5480(2)	10116(11)	6962(4)	17(3)
H(6)	5720(2)	10686(12)	7775(5)	26(4)
H(7A)	5400(2)	10607(13)	8589(5)	30(4)
H(7B)	6810(2)	9679(12)	8436(5)	25(4)
H(8)	4856(19)	8322(11)	8567(4)	17(3)
H(10)	4002(18)	7361(11)	9240(4)	14(3)
H(11A)	1070(2)	8826(11)	9390(5)	18(3)
H(11B)	870(2)	7537(12)	9394(5)	22(4)
H(12)	0(2)	8266(11)	8675(4)	18(3)
H(14)	3170(19)	8133(11)	7967(4)	14(3)
H(15A)	-250(2)	9175(13)	7845(5)	29(4)
H(15B)	90(2)	7901(12)	7899(5)	25(4)
H(16A)	-490(2)	8811(13)	7102(5)	31(4)
H(16B)	530(2)	7716(13)	7132(5)	30(4)
H(18)	620(2)	9714(11)	6515(4)	22(3)
H(19)	1310(2)	7543(12)	6373(5)	22(3)
H(20)	1580(2)	8701(12)	5524(5)	29(4)
H(21A)	4720(2)	8108(12)	5514(5)	29(4)
H(21B)	4480(2)	7574(14)	5995(6)	34(4)
H(22A)	5330(2)	9697(12)	5790(5)	21(3)
H(22B)	5600(2)	9143(12)	6264(5)	28(4)
H(24A)	780(2)	11011(13)	7022(6)	33(4)
H(24B)	1880(2)	11515(14)	7434(5)	34(4)
H(24C)	500(2)	10569(13)	7533(5)	34(4)
H(25A)	4700(2)	10021(12)	9768(5)	23(3)
H(25B)	2980(2)	10191(13)	9490(5)	32(4)
H(25C)	4740(2)	10716(14)	9294(5)	35(4)
H(26A)	7090(2)	8079(12)	9126(5)	27(4)
H(26B)	6930(2)	8689(11)	9622(5)	21(3)
H(26C)	7490(2)	9383(11)	9177(5)	21(3)
H(27A)	1120(2)	10252(12)	8898(5)	27(4)
H(27B)	220(2)	10157(14)	8421(5)	36(4)
H(27C)	2100(2)	10724(12)	8472(5)	24(4)
H(28A)	3500(3)	7485(15)	6802(6)	42(5)
H(28B)	4020(2)	7846(14)	7301(6)	37(4)
H(28C)	5000(3)	8377(15)	6898(6)	48(5)
H(29A)	-1500(2)	7651(13)	5971(5)	30(4)

H(29B)	-1320(2)	8880(13)	5928(6)	33(4)
H(29C)	-1680(2)	8333(13)	6433(6)	35(4)
H(30A)	1770(3)	6405(14)	5713(6)	41(5)
H(30B)	2290(2)	6910(12)	5223(6)	32(4)
H(30C)	200(2)	6972(13)	5426(5)	34(4)

7.3 ^{1}H and ^{13}C NMR spectra of compounds 85, 86, 101 and 104

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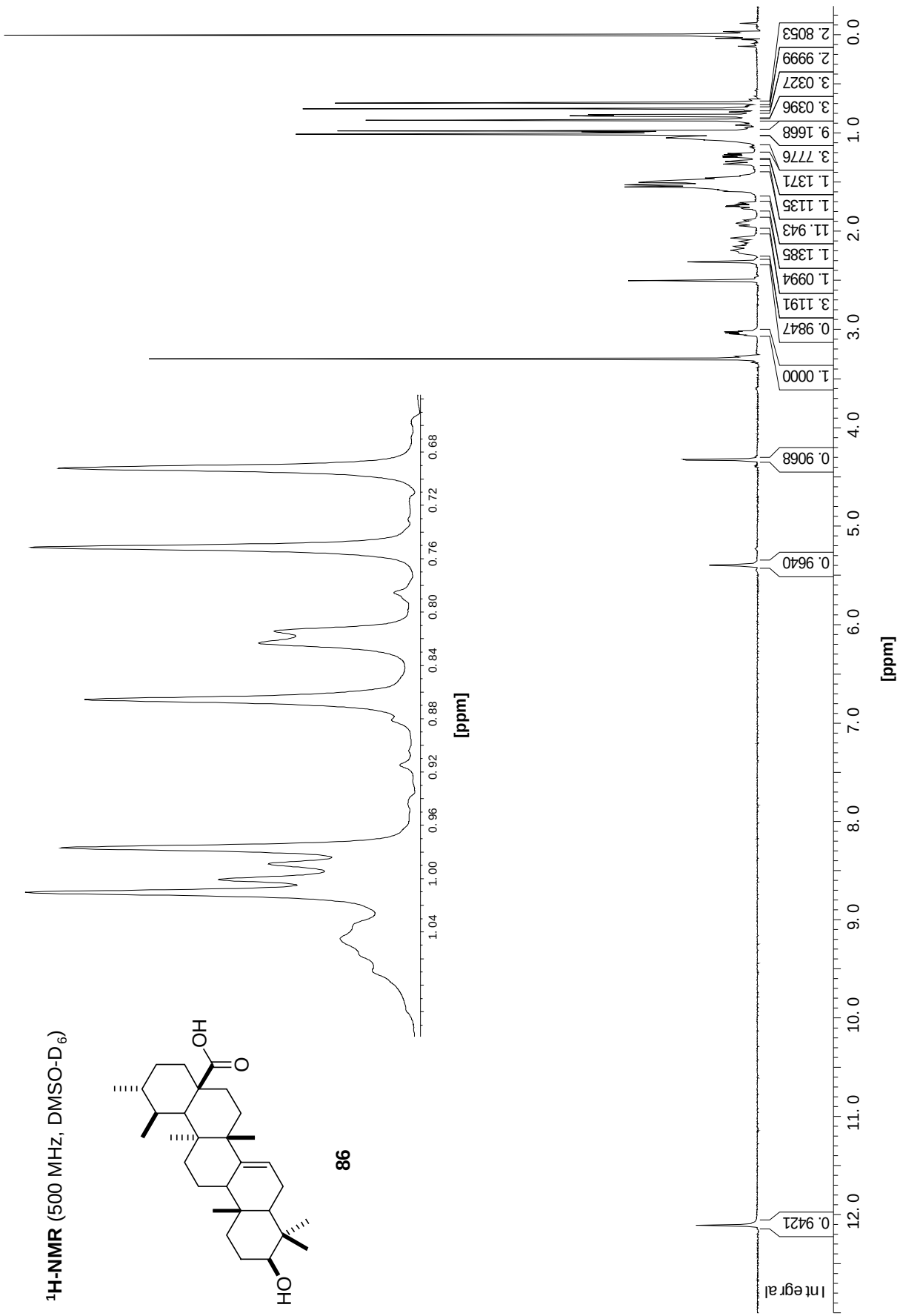
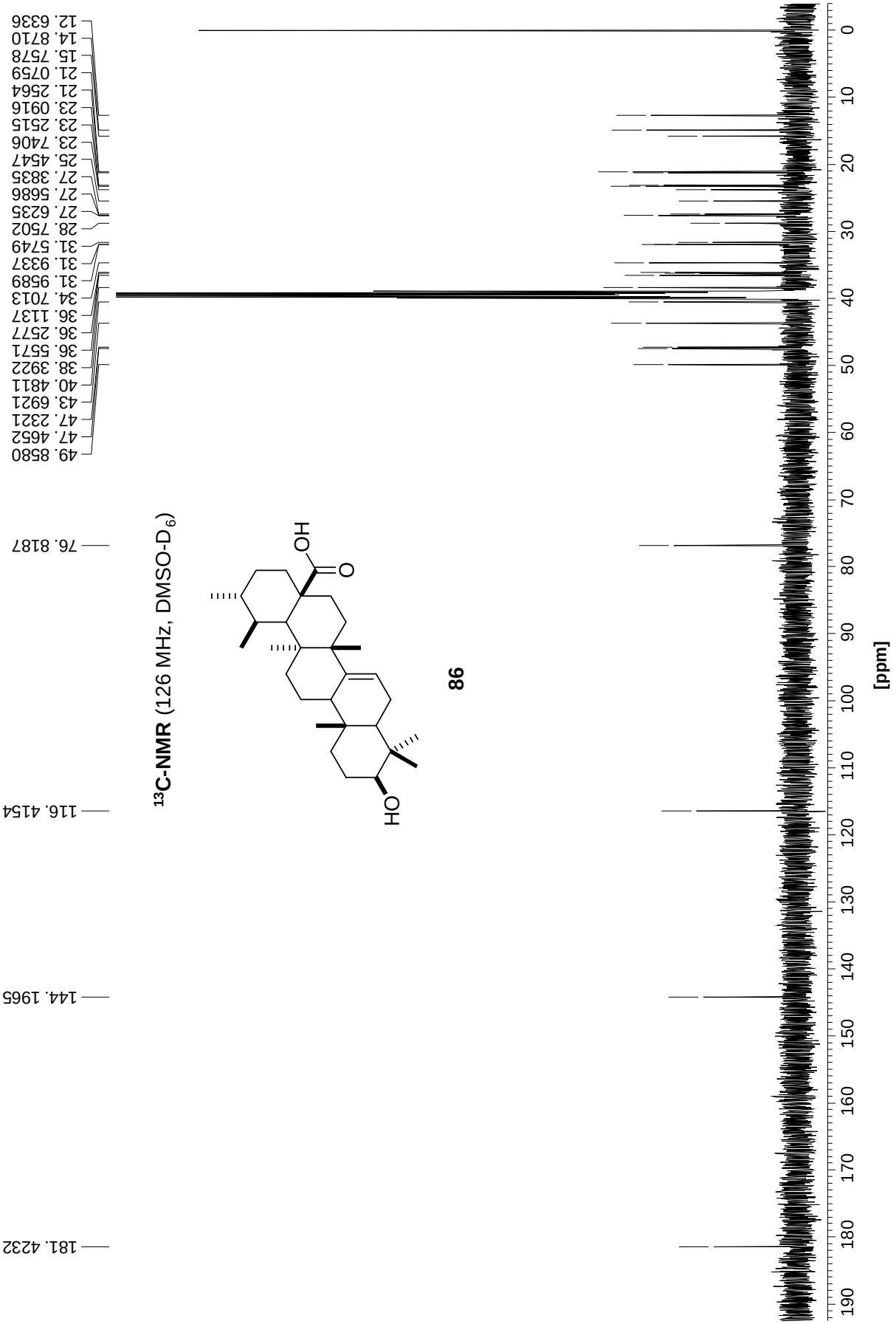
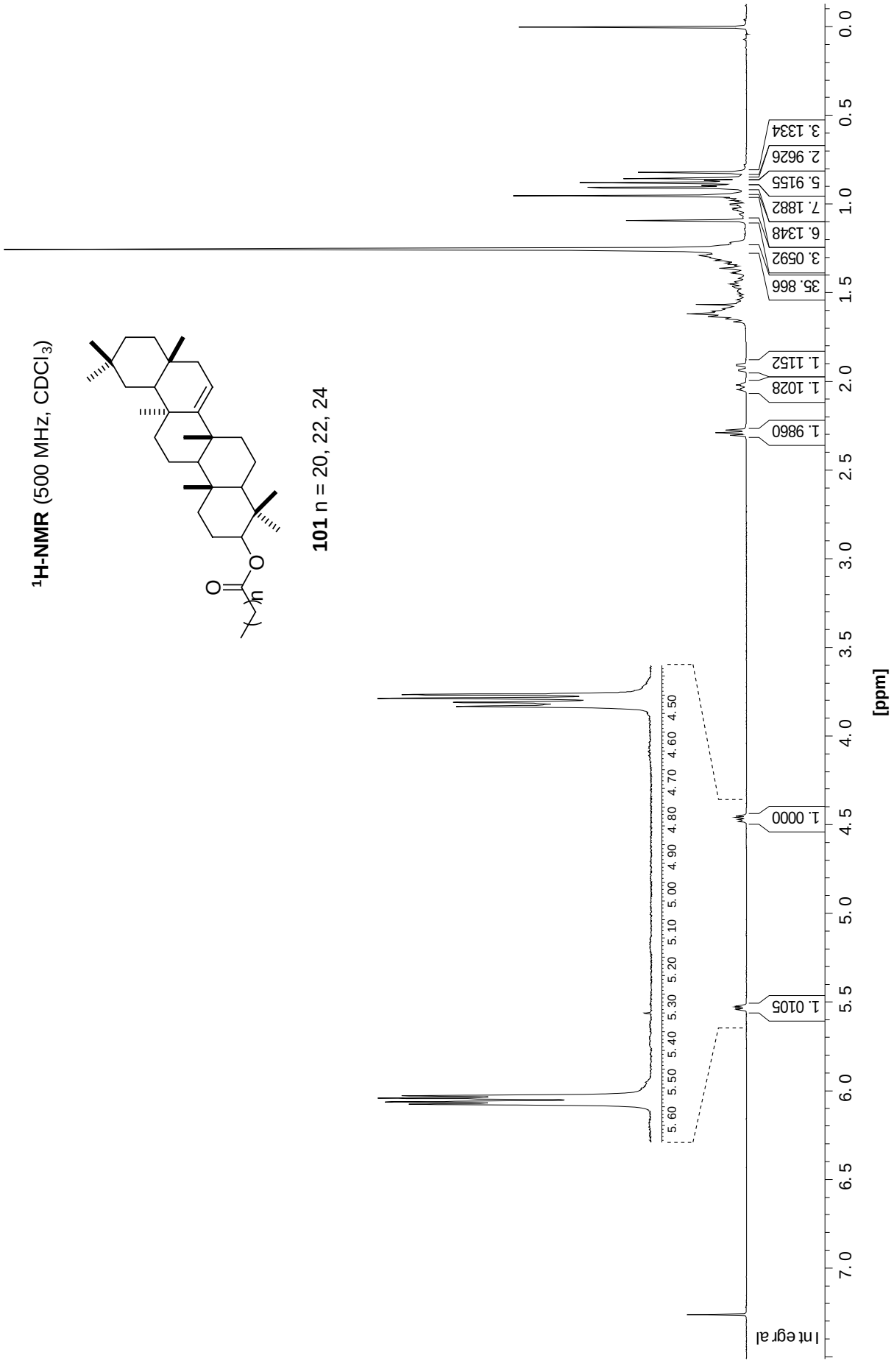
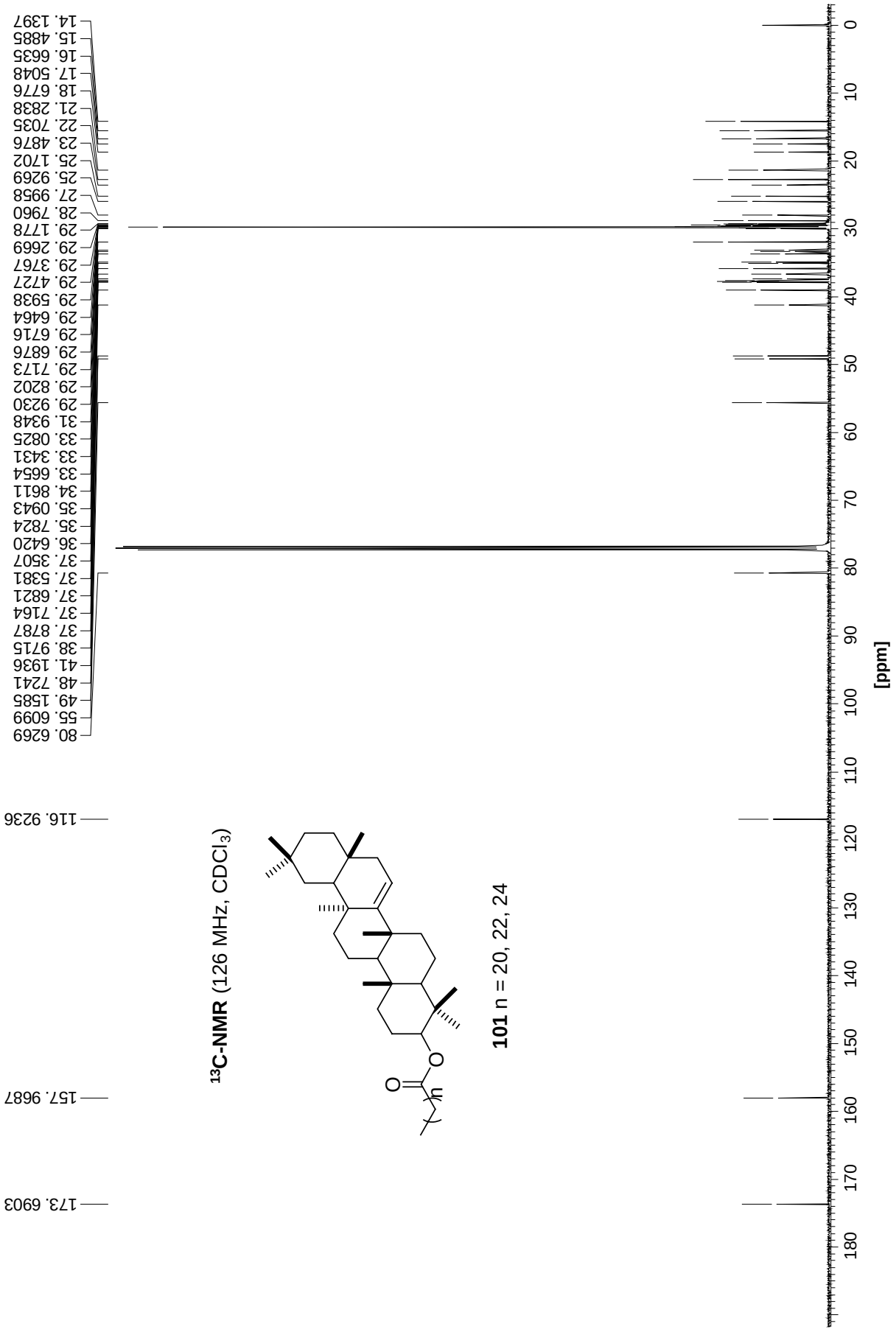
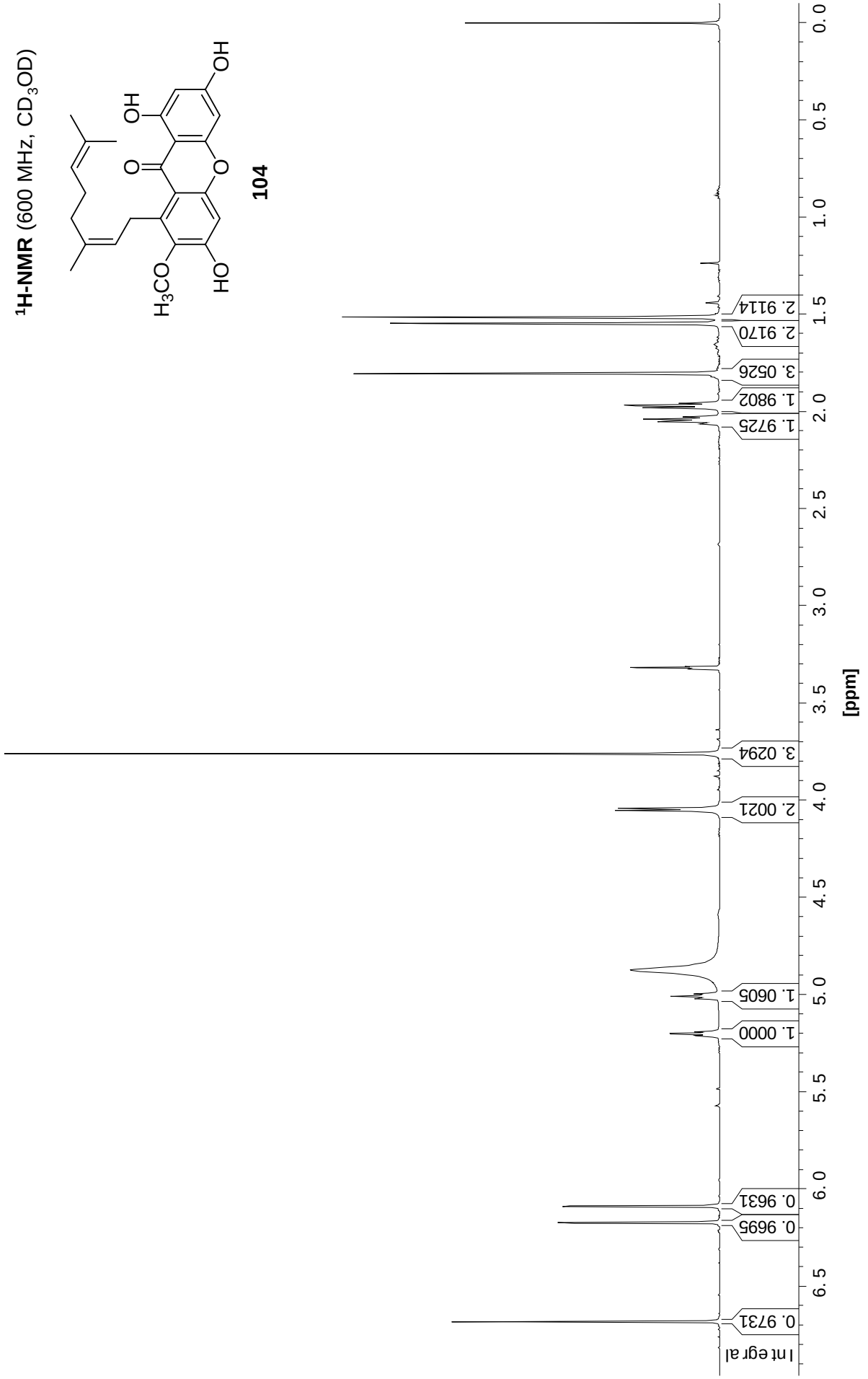
14.8340
14.9553
21.1299
21.2434
23.0472
23.2918
23.8239
25.5143
27.7956
28.8266
31.6087
32.0529
34.9641
36.2123
36.5821
38.4681
38.5679
40.6437
43.2321
43.7741
47.6048
70.8516
70.4310
115.2831
144.9745
181.4607
13C-NMR (63 MHz, DMSO-D6)
OH
O
OH
OH
85
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
[ppm]

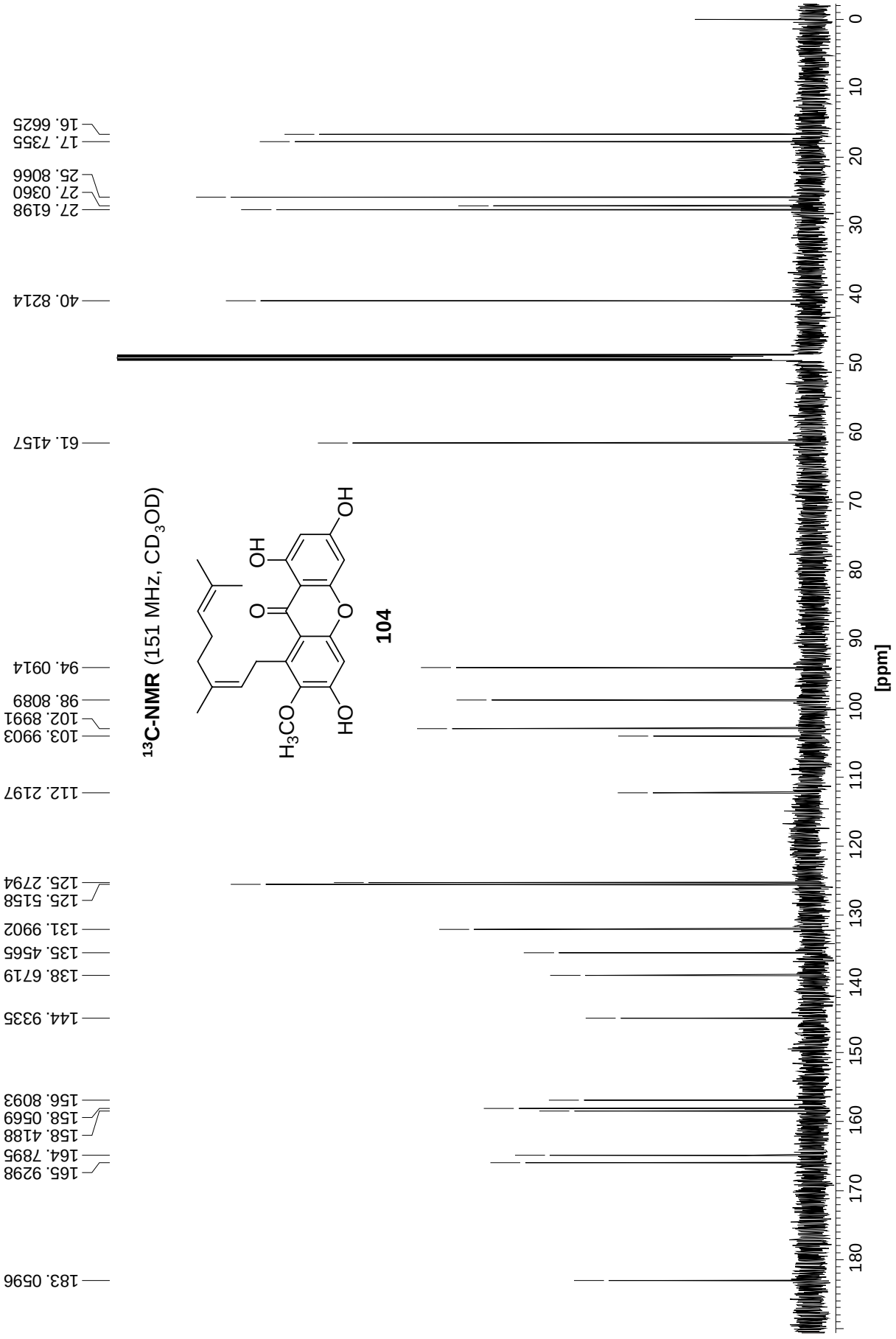

1H-NMR (500 MHz, DMSO-D6)
86
OH
O
OH
[ppm]
Integral
0.9421
0.9640
0.9068
1.0000
0.9847
3.1191
1.0994
1.1385
11.943
1.1135
1.1371
3.7776
9.1668
3.0396
3.0327
2.9999
2.8053


12.6336
14.8710
15.7578
21.0759
21.2564
23.0916
23.2515
23.7406
25.4547
27.3835
27.5686
27.6235
28.7502
31.5749
31.9337
31.9589
34.7013
36.1137
36.2577
36.5571
38.3922
40.4811
43.6921
47.2321
47.4652
49.8580
76.8187
116.4154
144.1965
181.4232
13C-NMR (126 MHz, DMSO-D6)
OH
O
HO
86
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
[ppm]


¹H-NMR (500 MHz, CDCl3)
101 n = 20, 22, 24
O
O
n
Integral
[ppm]
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
3.1334
2.9626
5.9155
7.1882
6.1348
3.0592
35.866
1.1152
1.1028
1.9860
1.0000
1.0105
4.50
4.60
4.70
4.80
4.90
5.00
5.10
5.20
5.30
5.40
5.50
5.60


14.1397
15.4885
16.6635
17.5048
18.6776
21.2838
22.7035
23.4876
25.1702
25.9269
27.9958
28.7960
29.1778
29.2669
29.3767
29.4727
29.5938
29.6464
29.6716
29.6876
29.7173
29.8202
29.9230
31.9348
33.0825
33.3431
33.6654
34.8611
35.0943
35.7824
36.6420
37.3507
37.5381
37.6821
37.7164
37.8787
38.9715
41.1936
48.7241
49.1585
55.6099
80.6269
116.9236
157.9887
173.6903
[ppm]
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
^{13}C-NMR (126 MHz, CDCl$_3$)
O
O
n
101 n = 20, 22, 24


¹H-NMR (600 MHz, CD3OD)
104
Integral
0.9731
0.9695
0.9631
1.0605
1.0000
2.0021
3.0294
1.9725
1.9802
3.0526
2.9170
2.9114
[ppm]


16.6625
17.7355
25.8066
27.0360
27.6198
40.8214
61.4157
94.0914
98.8089
102.8991
103.9903
112.2197
125.2794
125.5158
131.9902
135.4565
138.6719
144.9335
156.8093
158.0569
158.4188
164.7895
165.9298
183.0596
13C-NMR (151 MHz, CD3OD)
OH
OH
O
O
H3CO
HO
104
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
[ppm]

8 References

Abe, I.; Rohmer, M.; Prestwich, G. D. *Chemical Review*, **1993**, 93, 2189-2206.

Abisch, E.; Reichstein, T. *Helvetica Chimica Acta*; **1960**, 43, 1844-1861.

Adomako, D. *Journal of the Science of Food and Agriculture*, **1977**, 28, pp. 384-386.

African Regional Workshop, 1996. *Conservation and Sustainable Management of Trees,* project workshop held in Harare, Zimbabawe, July, **1996**.

Agarwal, K. P.; Rastogi, R. P. *Phytochemistry*, **1974**, 13, 2623-2645.

Agarwal, K. P., Roy, A. C.; Dhar, M. L. *Indian Journal of Chemistry*, **January 1963**, 1, 28-30.

Ageta, H.; Arai, Y. *Phytochemistry*, **1983**, 22, 1801-1808.

Agrawal, P. K. *Phytochemistry*, **1992**, 31, 3307-3330.

Agrawal, P. K.; Jain, D. C. *Progress in Nuclear Magnetic Resonance Spectroscopy*, **1992**, 24, 1-90.

Ainslie, J. R. List of plants used in Native Medicine in Nigeria. University of Oxford, imperial Forestry Institute, paper 7, **1937**

Akerele, O. M. D. Importance of Medicinal Plants. Nat. Resour. Hum. Health-Plants Med. Nutr. Value, Proc. WHO Symp. Plants Health All: Sci. Adv., 1st, **1992**, Meeting Date 1991, pp.66-91.

Alam, M. S., Chopra, N., Ali, M.; Niwa, M. *Phytochmistry*, **1996**, 41, 1197-1200.

Alves, T. M.; Nagem, T. J.; De Carvalho, L. H.; Krettli, A. U.; Zani, C. L. *Planta Medica*, **1997**, 63, 554-555.

Ampofo, S. A.; Waterman, P. G. *Phytochemistry*, 1986, 25, 2351-2355.

Anon. Bois et Forets des Tropiques, Mukulungu (*Autranella congolensis)*, **1954**, 36, pp 25-28.

Baker, D.; Mocek, U.; Garr, C. In *Biodiversity: New Leads for Pharmaceutical and Agrochemical Industries*; Wrigley, S. K., Hayes, M. A., Thomas, R., Chrystal, E. J. T., Nicholson, N., Eds.; The Royal Society of chemistry: Cambridge, UK, **2000**; pp 66-72.

Bamps, P. Flore du Congo, du Rwanda et du Burundi; **1970**, pp. 30-35.

Banerji, R.; Misra, G.; Nigam, S. K. *Indian Journal of Chemistry, Section B: Organic Chemistry Including Medicinal Chemistry*, **1979**, 18B(3), 293-294.

Barba, B., Diaz, J. G.; Herz, W. *Phytochemistry*, **1992**, 31, 4374-4375.

Barbey, S.; Goossens, L.; Taverne, T.; Cornet, J.; Choesmel, V.; Rouaud, C.; Gimeno, G.; Yannic-Arnoult, S.; Michaux, C.; Charlier, C.; Houssin, R.; Henichart, J. P. *Bioorganic & Medicinal Chemistry Letters*, **2002**, 12, 779-782.

Basu, N.; Rastogi, R. P. *Phytochemistry*, **1967**, 6, 1249-1270.

Battistini, B.; Botting, R.; Bakhle, Y. S. Drugs, News Perspect. 1994, 7, p 501.

Bennett, G. J.; Harrison, L. J.; Sia, G. L.; Sim, K. Y. *Phytochemistry*, **1993**, 32, 1245-1251.

Bensky, D.; Gamble, A. *Chinese Herbal Medicine; Materia Medica,* New Edition; Eastland Press: Seattle, **1993**.

Bhargava, K. P., Gupta, M. B., Gupta, G. P., Mitra, G. R. *Indian Journal of Medicine*, **1970**, 58, 724-730.

Bills, G.; Dombrowski, A.; Pelaez, F.; Polishook, J.; An, Z. In *Tropical Mycology: Micromycetes;* Watling, R., Frankland, J. C., Ainsworth, A. M., Issac, S., Robinson, C. H., Eds.; CABI Publishing: New York, **2002**, vol. 2, pp 165-194.

Binggeli P. The ecology of Maesopsis invasion and dynamics of the evergreen forest of the East Usambaras, and their implications for forest conservation and forestry practices. In A.C. Hamilton and R. Bensted-Smith (Eds). Forest conservation in the East Usambara mountains, Tanzania, **1989**, pp. 269-300. IUCN, Gland.

Binggeli, P. and Hamilton, A.C. Biological invasion by *Maesopsis eminii* in the East Usambara forests, Tanzania. Opera Bot., **1993**, 121, 229-235.

Boiteau, P., Rakoto, R. A., Paschi, B. *Les triterpenoides en physiologie vegetale et animale*. Paris: Gauthier-Villars, **1964a**, p. 412-415.

Boiteau, P., Rakoto, R. A., Paschi, B. *Les triterpenoides en physiologie vegetale et animale*. Paris: Gauthier-Villars, **1964b**, p. 415.

Boiteau, P., Rakoto, R. A., Paschi, B. *Les triterpenoides en physiologie vegetale et animale*. Paris: Gauthier-Villars, **1964c**, p. 602-622.

Bolza, E., and Keating, W. G. African Timbers: the properties, uses, and characteristics of 700 species. **1972**, CSIRO. Div. of Build. Res., Melbourne, Australia.

Brown, G.D. The biosynthesis of steroids and triterpenoids. *Natural Product Reports*, **1998**, 15, 653–696.

Borel, C.; Gupta, M. P.; Hostettmann, K. *Phytochemistry*, **1987**, 26, 2685-2689.

Budzikiewicz, H., willson, J. M.; Djerassi, C. *Journal of American Society*, **1963**, 85, 3688-3699.

Cambie, R. C.; Rutledge, P. S.; Wellington, K. D. *Journal of Natural Products*, **1997**, 60, 1303-1306.

Cameron, D. W.; Feutrill, G. I.; Keep, P. L. C. *Tetrahedron Letters*, **1989**,30, 5173-5176.

Caputo, R.; Lorenzo, M.; Pietro, M.; Previtera, L. *Phytochemistry*, **1974**, 13, 2825-2827.

Chakravarty, A. K., Das, B.; Mukhopadhyay, S. *Tetrahedron*, **1991**, 47, 2337-2350.

Chávez, Juceni P.; Dos Santos, Ihnamarck D.; Cruz, Frederico G.; David, Jorge M. *Phytochemistry*, **1996**, 41, 941-943.

Chemli, R., Babadjamian, A., Faure, R., Boukef, K., Balansard, G.; Vidal, E. *Phytochemistry*, **1987**, 26, 1785-1788.

Chudnoff, M. Tropical Timbers of the World. **1984**, USDA Forest Service. Ag. Handbook No. 607.

Cragg, G. M.; Newman, D. J.; Snader, K. M. *Journal of Natural Products*, **1997**, 60, 52-60.

Cumming, A. M.; Thomson, R. H. *Phytochemistry*, **1970**, 9, 2399-2400.

Curry, K. J. *American Journal of Botany*, **1987**, 74, 1332-1338.

Crublet, M.-L., Pouny, I., Delaude, C. *Journal of Natural Products*, **2002**, 65, 1560-1567.

Cryer, B. and Feldman, M. *American Journal of Medicine*, **1998**, 104, 413-421.

Dagne, E., Bekele, A.; Waterman, P. G. *Phytochemistry*, **1989**, 28, 1897-1900.

Dagne, E.; Steglich, W. *Phytochemistry*, **1984**, 23, 1729-1731.

Das, M. C.; Mahato, S. B. *Phytochemistry*, **1983**, 22, 1071-1095.

Devkota, P. K. Ph.D. Thesis, **2005**, H.E.J. Research Institute of Chemistry. International Center for Chemical Sciences, University of Karachi.

Djerassi, C.; Budzikiewicz, H.; Wilson, J. M. *Tetrahedron Letters*, **1962**, 3, 263-270.

Domon, B.; Hostettmann, K. *Helvetica Chimica Acta*, **1984**, 67, 131-1315.

Dubois, R. N.; Abramson, S. B.; Crofford, L.; Gupta, R. A.; Simon, L. S.; Van de Putte, L. B.; Lipsky, P. E. *FASEB*, **1998**, 12, 1063-1073.

Edwards R.; Gatehouse J. A. Secondary metabolism. In: Plant biochemistry and molecular biology. **1999**, New York: John Wiley & Sons. p. 193-218.

Ekpa, O., Anam, E.; Vethaviyasar, N. *Planta Medica*, **1985**, 528.

Eskander, J.; Lavaud, C.; Abdel-khalik, S. M.; Soliman, H. S. M.; Mahmoud, I. I.; Long, C. *Journal of Natural Products*, **2005**, 68, 832-841.

Fabricant, D. S.; Farnsworth, N. R. *Environmental Health Perspectives*, **2001**, 109, 69-75.

Facchini P. J.; Huber-Allanach K. L.; Tari L. W. *Phytochemistry*, **2000,** 54, 121-138.

Farnsworth, N. R. The role of medicinal plants in drug development. Natural products and drug development, Alfred Benzon Symposium 20. Eds P. Krogsgaard-Larson, S. Brogger Christensen, H. Munsgaard, Copenhagen **1984**.

Farnsworth, N. R.; Soejarto, D. D. *Ethnobotany*, **1985**, 39, 231-240.

Fokou, P. A.; Stamler, H. G.; Neumann, B.; Huber, T.; Lontsi, D.; Kemami, W. H. V.; Sewald, N. *Journal of Natural Products*, **2004**, 67, 2124-2126.

Forghani, F.; Ouellet, M., Keen, S., et al. *Analytical Biochemistry*, **1998**, 264, 216-221.

Gariboldi, P.; Verotta, L.; Gabetta, B. *Phytochemistry*, **1990**, 29, 2629-2635.

Geetha, T.; Varalakshmi, P. *Fitoterapia*, **1988**, 79, 13-19.

Gonzalez, A. G.; Ferro, E. E. and Ravelo, A. G. *Phytochemistry*, **1987**, 26, 2785-2788.

Goodwin, T. W. In Biosynthesis of isoprenoid compounds. Porter, J. W., Spurgeon, S. L. Eds.; Wiley: New York, **1980**, Vol.1, pp. 443-480.

Goodwin, T. W.; Mercer, E. I. Introduction to plant biochemistry. Second Edition. Pergamon Press, Oxford, England, **1983**.

Gopalakrishnan, G.; Banumathi, B.; Suresh, G. *Journal of Natural Products*, **1997**, 60, 519-524.

Gopalakrishnan, C.; Shankaranarayanan, D.; Kameswaran, L.; Nazimudeen, S. K. *Indian Journal of Experimental Biology*, **1980**, 18, 843-846.

Grabley, S.; Thiericke, R. Drug Discovery from Nature. Springer-Verlag, Berlin: Heidelberg, **1999**, pp. 3-37.

Gulcin, I.; Kufrevioglu, O. I.; Oktay, M.; Buyukokuroglu, M. E. *Journal of Ethnophamacology*, **2004**, 90, 205-215.

Gunasekera, S. P.; Sivapalan, K.; Sultanbawa, M. U. S.; Ollis, W. D. *Journal of the Chemical Society, Perkin Transactions 1: Organic and Bio-Organic Chemistry*, **1977a**, 1, 11-14.

Gunasekera, S. P.; Sultanbawa, M. U. S. *Journal of Chemical Society, Perkin Transactions 1*, **1977b**, 6-10.

Hallé, N. Flore du Gabon – Rhamnacée; Museum National d'Histoire: Paris V^{e}, 1970 ; pp 49-54.

Halliwell, B.; Gutteride, J. M. C. *Molecular aspects of Medicine*, **1985**, 8, 89-93.

Hambert, M.; Samuelsson, B. *Proceedings of the National Academy of Sciences U.S.A.*, **1973**, 70, 899-903.

Harborne, J. B.; Baxter, H. "Phytochemical Dictionnary and Hand book of Bioactive Compounds from Plants." **1993**, Taylor and Francis, London.

Hashimoto, F., Kashiwada, Y., Cosentino, L. M., Chen, C.-H., Garrett, P. E. and Lee, K.-H. *Bioorganic & Medicinal chemistry*, **1997**, 5, 2133-2143.

Hiramoto, M.; Wanatabe, K. *Journal of Pharmaceutical Society Japan*, **1939**, 59, 261.

Holt, H-G.; Schriefers, H. *Hoppe-Seylers Z. Physiol. Chem.*, 1973, 354, 507-513.

Hostettmann, K.; Marston, A. Saponins-Chemistry and Pharmacology of Natural Products. Cambridge University Press; **1995a**; pp 1-2.

Hostettmann, K.; Marston, A. Saponins-Chemistry and Pharmacology of Natural Products. Cambridge University Press; **1995b**; pp 18-20.

Hostettmann, K.; Marston, A. Saponins-Chemistry and Pharmacology of Natural Products. Cambridge University Press; **1995c**; pp 143-174.

Hostettmann, K.; Marston, A. Saponins-Chemistry and Pharmacology of Natural Products. Cambridge University Press; **1995d**; p 185

Hostettmann, K.; Marston, A. Saponins-Chemistry and Pharmacology of Natural Products. Cambridge University Press; **1995e**; p 177

Hostettmann, K.; Marston, A. Saponins-Chemistry and Pharmacology of Natural Products. Cambridge University Press; **1995f**; pp 175-231.

Hostettmann, K.; Marston, A. Saponins-Chemistry and Pharmacology of Natural Products. Cambridge University Press; **1995g**; pp 239-286.

Hutchinson, J.; Dalziel, J. M. Flora of West Tropical Africa; **1963a**, Vol. II, pp 20-21.

Hutchinson, J.; Dalziel, J. M. Flora of West Tropical Africa; **1963b**, Vol. II, pp.290-291.

Hui, W. H.; Li, M. M. *Journal of Chemical Society Perkin transactions*, **1976**, 1, 23-30.

Iinuma, M.; Tosa, H.; Tanaka, T.; Asai, F.; Kobayashi, Y.; Shimano, R.; Miyauchi, K. I. *Journal of Pharmacy and Pharmacology*, **1996**, 48, 861-865.

Imre, S. *Zeitschrift für Naturforschung*, **1973**, 28C, 436-439.

Ikan, R. Natural products-A Laboratory Guide, 2nd ed.; Academic Press; San Diego, **1991a**; p 56.

Ikan, R. Natural products-A Laboratory Guide, 2nd ed.; Academic Press; San Diego, **1991b**; p 138-139.

Irvine, F. R. Woody plants of Ghana; Oxford University Press: London, **1961**, pp 480-481.

Ito, J.; Chang, F.; Wang, H.; Park, Y. K.; Ikegadi, M.; Kilgore, N.; Lee, K. *Journal of Natural Products*, **2001**, 64, 1278-1281.

Itoh, T.; Kikuchi, Y.; Tamura, T.; Matsumoto, T. *Phytochemistry*, **1981**, 20, 761-764.

Jackson, B.; Locksley, H. D.; Scheinmann, F. J. Journal of Chemical Society ©, **1967**, 2500-2507.

Jahan, N.; Ahmed, W.; Malik, A. *Journal of Natural Products*, **1995**, 58, 1244-1247.

Jammula, S. R.; Pepalla, S. B.; Telikepalli, H.; Rao, K. V. J.; Thomson, R. H. *Phytochemistry*, **1991**, 30, 2103-2104.

Janes, N. F.; King, F. E.; Morgan, J. W. W. *Chemistry and Industry*, **1961**, 346.

Jones, W. D.; Albrecht, W. L.; Munro, N. L.; Stewart, K. T. *Journal of Medicinal Chemistry*, **1977**, 20, 594.

Joshi, B. S., Ramanathan, S.; Venkataraman, K. *Tetrahedron Letters*, **1962**, 3, 951-955.

Karliner, J. and Djerassi, C. *Journal of Organic Chemistry*, **1966**, 31, 1945-1956.

Kashiwada, Y.; Wang, H. K.; Nagao, T.; Kitanaka, S.; Yasuda, I.; Fudjioka, T.; Yamagishi, T.; Cosentino, L. M.; Kozuka, M.; Okabe, H.; Ikeshiro, Y.; Hu, C. Q.; Yeh, E.; Lee, K. H. *Journal of Natural Products*, **1998**, 61, 1090-1095.

Kawanishi, K. and Hashimoto, Y. *Phytochemistry*, **1987**, 26, 749-752.

Kerharo, J.; Bouquet, A. Plantes medicinales et toxiques de la côte d'Ivoire – Haute Volta. Ministère de la France d'Outre-Mer. Office de la Recherche Scientifique Outre-Mer, paris, **1950**, p 291

Knut, D., Aksnes, D. W.; Francis, G. W. *Magnetic Resonance in Chemistry*, **1992**, 30, 359-363.

Kochetkov, N. K.; Khorlin, A. J. *Arzneimittelforsch.*, **1966**, 16, 101-109.

Koizumi, N., Fujimoto, Y., Takeshita, T.; Ikekawa, N. *Chemical and Pharmaceutical Bulletin*, **1979**, 27, 38-42.

Kojima, H.; Ogura, H. *Phytochemistry*, **1989**, 28, 1703-1710.

Kozak, Kevin R.; Prusakiewicz, Jeffery J.; Rowlinson, Scott W.; Marnett, Lawrence J. *Bioorganic & Medicinal Chemistry Letters*, **2002**, 12, 1315-1318.

Laine, R.A.; Elbein, A.D. *Biochemistry*, **1971**, 10, 2547-2553.

Lavaud, C., Massiot, G., Becchi, M., Misra, G., Nigam, S. K. *Phytochemistry*, **1996**, 41, 887-893.

Lee, H. H., Chan, H.-K. *Phytochemistry*, **1977**, 16, 2038-2040.

Lewis, K. G.; Tucker, D. J. *Australian Journal of Chemistry*, **1983**, 36, 2297-2305.

Likhitwitayawuid, Kittisak; Phadungcharoen, Thatree; Mahidol, C.; Ruchirawat, S. *Phytochemistry*, **1997,** 45, 1299-1301.

Lin, L., Chou, C.; Kuo, Y. *Journal of Natural Products*, **2001**, 64, 674-676.

Lipsky, P. E. *American Journal of Medicine*, **1999**, 106, 51S-57S

Lombardino, J. G. In Nonsteroidal antiinflammatory Drugs; Wiley-Interscience/John Wiley & Sons: New York, **1985**.

Mahato, S. B.; Kundu, A. P. *Phytochemistry*, **1994**, 37, 1517-1575.

Mahato, S. B., Nandy, A. K.; Roy, G. *Phytochemistry*, **1992**, 31, 2199-2249.

Matsumoto, K.; Akao, Y.; Kobayashi, E.; Ohguchi, Kenji; Ito, T.; Tanaka, T.; Iinuma, M.; Nozawa, Y. *Journal of Natural Products*, **2003**, 66, 1124-1127.

Maxwell, S. R. *Drugs*, 1995, 49, 345-361.

Meksuriyen, D.; Nanayakkara, N. P. D.; Phoebe, C. H. Jr.; Cordell, G. A. *Phytochemistry*, **1986**, 25, 1685-1689.

Misra, G., Nigam, S. K., Mitra, C. R. *Planta Medica*, **1974**, 26, 155-165.

Misra, G., Nigam, S. K., Mitra, C. R. *Phytochemistry*, **1970**, 9, 2067-2068.

Monache, D. F.; Mac-Quhae, M. M.; Monache, D. G.; Bettolo, M. G. B.; Lima, R. A. *Phytochemistry*, **1983**, 22, 227-232.

Morel, C., Hay, A. E. Litaudon, M., Sévenet T., Seraphin, D., Bruneton, J.; Richomme, P. *Molecules*, **2002**, 7, 38-50.

Nay, B., Collet, M., Lebon M., Chèze, C.; Vercauteren, J. *Tetrahedron Letters*, **2002**, 43, 2675-2678.

Nay, B., Monti, J. P., Nuhrich, A., Deffieux, G., Mérillon, J. M.; Vercauteren J. *Tetrahedron Letters*, **2001**, 41, 9049-9051.

Newman, D. J.; Cragg, G. M.; Snader, K. M. *Journal of Natural Products*, **2003**; 66, 1022-1037.

Newman, D. J.; Cragg, G. M.; Snader, K. M. *Natural Product Reports*, **2000**, 17, 215-234.

Ngounou, F. N.; Lontsi, D.; Sondengam B. L. *Phytochemistry*, **1988**, 27, 2287-2289.

Nguyen, L. H. D.; Harrison, L. J. *Phytochemistry*, **1998**, 50, 471-476.

Nkengfack, E. A.; Azebazé, G. A.; Vardamides, J. C.; Fomum, T. Z.; Van Heerden, F. R. *Phytochemistry*, **2002**, 60, 381-384.

Normand, D. Sur le *Maesopsis* de l'ouest africain et le bois de nkanguele. Revue Botanique Appliquée. Agriculture Tropicale, **1935**, 15, pp.252-263.

N'Sosso, D. *in litt*. N'Sosso contributions to *the Conservation and Sustainable Management of Trees,* project for the Congo, **1995**.

Noumi, E.;Tchakonang, N. Y. V. *Journal of Ethnopharmacology*, **2001**, 76, 263-268.

Öksüz, S.; Topcu, G. *Phytochemistry*, **1987**, 26, 3082-3084.

Oomah, B.D. Isolation, Characterization and Assessment of Secondary Metabolites from Plants for Use in Human Health. PBI Bulletin, **2003**, Issue 1

Orsler, R.J. Personal communication to JC Mitchell from Forest Products Research Laboratory, Princes Risborough, Bucks, England, **1973**.

Patra, A.; Mitra, A. K.; Ghosh, A.; Barua, A. K. *Organic Magnetic Resonance*, **1981**, 15, 399-400.

Pattalung, P. N.; Thongtheeraparp, W.; Wiriyachitra; P.; Taylor, W. C. *Planta Medica*, **1994**, 60, 365-368.

Penning, T. M. *Journal of Pharmaceutical sciences*, **1985**, 74, 651-654.

Plockin, M. J. Traditional Knowledge of Medicinal Plants-the Search for New Jungle Medicine, In Conservation of Medicinal Plants (Akerele, O.; Heywood, V. and Synge H. eds), Cambridge University Press, Cambridge, UK, **1991**, pp 43-64.

Pooley, E. *The complete field guide to trees of Natal , Zululand and Transkei* . Natal Flora Publications Trust, **1993**, Durban.

Reynolds, W. F.; McLean, S.; Poplawski, J.; Enriquez, R. G.; Escobar, L. I.; Leon, I. *Tetrahedron*, **1986**, 42, 3419-3428.

Rosenthaler, L. *Pharmaceutische Zeitung*, **1928**, 73, 1307.

Rubinstein, I.; Goad, J. L.; Clague, A. D. H.; Mulheirn, L. J. *Phytochemistry*, **1976**, 15, 195-200.

Safayhi, H.; Sailier, E. R. *Planta Medica*, 1997, 63, 487-493.

Sahu, N. P. *Phytochemistry*, **1996**, 41, 883-886.

Sahu, N. P.; Koike, K.; Jia, Z.; Nikaido, T. *Tetrahedron*, **1995**, 51, 13435-13446.

Sakai, S.; Katsura, M.; Takayama, H.; Aimi, N.; Chokethaworn, N.; Suttajit, M. *Chemical and pharmaceutical Bulletin*, **1993**, 41, 958-960.

Sakakibara, J.; Kaiya, T.; Fukuda, H.; Ohki, T. *Phytochemistry*, **1983**, 22, 2553-2555.

Sakurai, N.; Yaguchi, Y.; Inoue, T. *Phytochemistry*, **1987**, 26, 217-219.

Santesson, C. G. Einige Drogen aus dem Kamerun-Gebiete und ihre einheimische Verwendung; *Archiv für Botanik*, **1926**, 20, 1-34.

Sen, S.; Sahu, N. P.; Mahato, B. S. *Phytochemistry*, **1995**, 38, 205-207.

Seo, E.-K.; Kim, N.-C.; Wani, M. C.; Wall, M. E.; Navarro, H. A.; Burgess, J. P.; Kawanishi, K.; Kardono, L. B. S.; Riswan, S.; Rose, W. C.; Fairchild, C. R.; Farnsworth, N. R. and Kinghorn, A. D. *Journal of Natural Products*, **2002**, 65, 299-305.
Seo, S.; Tomita, Y.; Tori, K.; Yoshimura, Y. *Journal of American Chemical Society*, **1978**, 100, 3331-3339.

Shah, P. J.; Gandhi, M.; S., Shah, M. B.; Goswami, S. S.; Santani, D. *Journal of Ethnopharmacology*, **2003**, 89, 305-311.

Shiojima, K.; Arai, Y.; Masuda, K.; Takase, Y.; Ageta, T.; Ageta, H. *Chemical and Pharmaceutical Bulletin*, **1992**, 40, 1683-1690.

Sia, G. L.; Bennett, G. J.; **Harrison, L. J.** *Phytochemistry*, **1995**, 38, 1521-1528.

Sica, D. and Zollo, F. *Tetrahedron Letters*, **1978**, 9, 837-838.

Simoneau, B.; Brassard, P. *Tetrahedron*, **1988**, 24, 1015-1022.

Sksamrarn, S.; Suwannapoch, N.; Ratananukul, P.; Aroonlerk, N.; Suksamrarn, A. *Journal of Natural Products*, **2002**, 65, 761-763.

Somanathan, R.; Sultanbawa, M. U. S. *Journal of Chemical Society, Perkin Transaction 1*, **1972**, 1935-1943.

Sreenivasan, M. S.; Dharmaraj, P. S. *Maesopsis eminii* Engl.- a fast growing shade tree for coffee. *Indian Coffee*, **1991**, 55, 17-20.

Srivastava, M.; Singh, J. *International Journal of Pharmacology*, **1994**, 32, 197-200.

Strobel, G.; Daisy, B.; Castillo, U.; Harper, J. *Journal of Natural Products*, (Review); **2004**; 67, 257-268.

Sultanbawa, M. U. S. *Tetrahedron*, **1980**, 36, 1465-1506.

Tandon, M., Yogendra, N. S.; Ragunath, S. T. *Phytochemistry*, **1990**, 29, 2957-2959.

Tomkins, G. *Journal of Biological Chemistry*, **1956**, 218, 437-447.

Tomkins, G. in "Recent Progress in Hormone Research," **1956a**, vol. 12, Pincus, G. Ed.; academic Press: New York, p 125

Tursch, B.; Savior, R.; Ottinger, R.; Chiurdoglu, G. *Tetrahedron Letters*, **1967**, 6, 539-543.

Vane, J. R. *Nature: New Biology*, **1971**, 231, 232-235.

Vane, J. R. *Inflammation Research*, **1998**, 47, S77.

Vane, J. R.; Bakhle, Y. S.; Botting, R. M. *Annual Review of Pharmacology and Toxicology*, **1998**, 38, 97-120.

Vane, J. R. and Botting, R. M. *International Journal of Tissue Reactions*, **1998**, 20, 3-15.

Verpoorte, R. *Journal of pharmacy and pharmacology*, **2000**, 52, 253-262.

Wagner, H.; Muesmann, J.; Lampart, J. B. *Zeitschrift für Untersuchung der Nahrungs- und Genussmittel sowie Gebrauuchsgegenstaende*, **1914**, 28, 244-249.

Walker, A. Articles in Bulletin de l'Institut d'Etudes Centrafricaines, Nos. 4, 5, 6 ; Usages Pharmaceutiques des plantes spontanée du Gabon, **1953**.

Walker, A. R., Sillans, R., Trochain, J. L. Plantes Utiles du Gabon, Ed. Paul Chevalier 12- Rue de Tournon Paris VI, **1961,** 354-355.

Wani, M. C.; Taylor, H. L.; Wall, M. E.; Goggon, P.; McPhail, A. T. *Journal of American Chemical Society*, **1971**, 93, 2325-2327.

Wansi, J. D. Contribution à l'étude phytochimique de 2 plantes medicinales du Cameroun: *Gambeya africana* (Sapotaceae) et *Drypetes molunduana* (Euphorbiaceae), Thèse de doctorat 3è cycle, **2000**, Université de Yaoundé.

Wink, M. Functions of Plant Secondary Metabolites and their Exploitation in Biotechnology. CRC Press, **1999**

Wendt, K. U.; Schulz, G. E., Corey, E. J.; Liu, D. R. *Angewandte Chemie International Edition*, **2000**, 39, 2812-2833.

WPS (White Point Systems): About Natural Products. http://www.wps2.com/aboutnp.htm

Yasue, M. *Yakugaku Zasshu*, **1970**, 90, 341-343.