The Chemical Constituents of

*Ehretia rigida, Apodytes dimidiata*

and *Ocotea kenyensis*

by

Trevor Steyn

B.Sc. Hons (Natal)

A thesis submitted in partial fulfillment
of the requirements of Master of Science
University of Natal.
Declaration

I hereby certify that this is a result of my own investigation and has not already been accepted in substance for any other degree and is not being submitted in canditure for any other degree.

............................

Trevor Steyn

I the undersigned, hereby certify that the above statement is true and correct.

............................

Professor S. E. Drewes (Supervisor)

Department of Chemistry and Chemical Technology
University of Natal
Pietermaritzburg
December 14, 1998
Dedication

I would like to dedicate this thesis to all the carbon, oxygen and hydrogen atoms that are giving up their valuable time to create a medium on which to present this work. Without them this thesis would have been impossible.
Acknowledgement

I would like to thank my supervisor, Professor S. E. Drewes, for his guidance and encouragement throughout the course of this project.

Thanks are also due to:
Mr. M. Watson and Mr. G. Crawley and his technical staff.
Dr T. Clarke and Ms. L. Smith for conducting trypanosome tests.
Professor C. C. Appleton and his assistants for molluscicidal activity tests.
Mr. P. Forder for glassblowing
My laboratory colleagues for helpful suggestions and idle banter

Special thanks are due to my wife and children, Kieran, Kim and “Hannah the Biblical Palindrome” for putting up with me during the course of this work.
Abstract.

Three species have been investigated in this project:

Iso-ocobullenone (p 23) and its probable precursor (p 27) have been isolated from the leaves and bark of *Ocotea kenyensis*. This is only the second time that these compounds have been isolated and the first time from this species. The isolation of these two compounds in the leaves establishes a commercially important chemical link with the bark. The bark of the closely related *Ocotea bullata* is the most sought after source of “muthi” in the Kwa-Zulu Natal region and it is becoming a very scarce commodity.

The leaves of *Ocotea kenyensis* have also yielded related compounds including Δ⁸-3,5-dimethoxy-3',4'-methylenedioxy-1',2',3',4'-tetrahydro-6'-oxo-7,1',8,3'-neolignan which is described here for the first time. Other compounds isolated were bacterialprenol, triacontane, β-sitosterol and a previously undescribed isoprenoid.

An investigation into the molluscicidal potential of compounds in *Apodytes dimidiata* was extended with a view toward control of the population of the intermediary snail host for bilharzia-causing trypanosomes. This study afforded the known compounds squalene, lupeol, betulinic acid and catechin. The synthesis of a previously isolated compound, 4-ethyl catechol, with molluscicidal activity was successfully completed and its structure and activity were confirmed.

A bio-assay guided isolation procedure was used to determine the potential value of compounds in *Ehretia rigida* for the control of sleeping sickness. Allantoin, α- and β-amyrin, triacontanol and β-sitosterol were isolated. Activity of the extracts was not high enough to be of commercial value.
Chapter 1 – Introduction

1.1 Introduction .............................. 1
1.2 A Topical Example ...................... 5
1.3 Natural Products work done in Kwa-Zulu Natal in the Last Five Years .............................. 11
   1.2.1 Drewes .................................. 11
   1.2.2 Mulholland .............................. 14
   1.2.3 Malan .................................. 17
   1.2.4 Pegel .................................. 20

Chapter 2 – Discussion

2.1 Ocotea kenyensis ......................... 24
   2.1.1 Iso-ocobullenone ....................... 24
   2.1.2 \( \Delta^8 \)-3,4,5-trimethoxy-3',6'-dihydro-3',4'-methylenedioxy-6'-oxo-8,3'-neolignan .............................. 27
   2.1.3 New compounds related to iso-ocobullenone .............................. 30
   2.1.4 Isoprenoids ............................. 35
   2.1.5 Other Constituents ...................... 37

2.2 Apodytes dimidiata ...................... 39
   2.2.1 Molluscicidal potential ................. 39
   2.2.2 Triterpenoids .......................... 41
   2.2.3 Other Constituents ...................... 44
   2.2.4 Synthesis of 4-ethyl catechol .......... 45
   2.2.5 Molluscicidal properties of 4-ethyl catechol .............................. 51
Chapter 3 – Experimental

3.1 Instrumentation

3.2 Chemicals

3.3 Extraction of Plant Material

3.4 Extractives of Ocotea kenyensis

3.5 Extractives of Apodytes dimidiata

3.6 Extractives of Ehretia rigida

3.7 Synthesis of 4-ethyl catechol

Chapter 4 – Bibliography

Chapter 5 – Spectra
Chapter 1

Introduction

1.1: Introduction

In 1992 the Earth summit was held in Rio de Janeiro. One hundred and fifty two of the one hundred and fifty three countries represented agreed that, “beyond personal interest, the health of the planet and conservation of its resources, particularly its biodiversity, were the most important factors for the long term survival of the human race.”[1]

The signatories are now bound to the development of national programmes for the conservation and sustainable use of biodiversity but perhaps the most important outcome of the summit is the rapid and encompassing increase in public awareness [2]. This has lead the governments of these less developed countries to the understanding that biodiversity is a valuable resource, that a country has the right to protect access to it and, if it allows access, to demand compensation [3].

Biodiversity is now firmly established as an asset and South Africa is rich in this respect. Approximately 23 000 plants, mostly endemic, occur within South Africa’s borders, which represents nearly 10% of all plants known to man [4]. Kwa-Zulu Natal
alone is home to more than 750 species of indigenous trees, more than two thirds of the country’s resource. This represents more than 11 times the number of indigenous tree species contained in the whole of Europe [5]. Natural product chemists in the country have a great natural resource but one that will not last: free access to this wealth of plants and the compounds that they contain.

The area of indigenous vegetation in South Africa is decreasing dramatically. The major reasons for this are: the spread of urban development, commercial and subsistence agriculture and exploitation of forests for timber [6]. The effect of reduction of forest area is particularly significant as forests cover only 0.3% of South Africa and are the source of over 130 species used as traditional medicines [7]. It has been estimated that over 90% of coastal lowlands forest has been lost as a result of sugar cane cultivation alone [8]. The commercial harvesting of medicinal plants is also of concern as indigenous species are being ‘mined’ on an exponentially increasing scale and in a completely unsustainable and wasteful fashion. Gatherers collect plant material for sale to urban street marketers who sell to sangomas and nyangas who dispense the plant parts to the public. Lack of storage facilities results in a large proportion of the gatherers’ harvests becoming spoiled and unusable before they ever reach the marketers. The higher prices paid by the urban medicinal plant markets for rare plants, compounds the threat to these species as they are then more sought after by gatherers. A high percentage of the public uses plants as medicine. Only 33% of a group of 271 respondents did not consult nyangas. Nearly 100% of a random sample of hospital patients used medicinal plants [9]. The radical increase in the population of the country has resulted in a proportionately escalated demand for medicinal plants but the supply is dwindling. Many species that were cheap and readily available in the past are not found in markets or are very expensive. The extinction of species with medicinal value would be a sore loss. In an attempt to preserve the indigenous plant diversity in South Africa, conservancies like Silver Glen nature reserve have been established. Funding for this project was supplied by the Durban city council. This 250-hectare operation just south of Durban holds stocks of rare species, in the wild and in a large nursery, to maintain a healthy genetic base. Courses are also run on plant propagation for sangomas and nyangas. In this way it is hoped that a degree of sustainability will be attained in the foreseeable future.
The huge diversity of species leads to the expectation that many therapeutically worthwhile compounds remain undiscovered; but to screen large numbers of plants and their compounds in a systematic way is vastly expensive. The use of folk beliefs and traditional healers as a short cut to the discovery and isolation of pharmacologically active compounds has been a productive approach. Virtually all currently used drugs derived from plants; including reserpine, quinine, digoxin, digitoxin, morphine, and codeine were discovered via this lead [10]. Plants are the primary source of medicinal treatment for 80% of the world’s population [2]. It is therefore not surprising that most countries have a well-established system of traditional medicine. Some indigenous healing systems are well documented and even commercially available but in other cases, such as in Kwa-Zulu Natal, traditional remedies are closely guarded secrets passed on solely to tribal apprentices [6]. The breakdown of tribal structure, as Westernisation becomes more prominent, results in fewer properly trained traditional healers and more untrained healers and diviners practising, particularly in urban areas. Until the late 1980s there was limited interest, in developed countries, in this hard earned indigenous knowledge and the rate at which it is disappearing [2]. The insurgence of a new area of science, namely ethnobotany, has begun to address this problem. Funding from large pharmaceutical companies and the concerted efforts of a few individuals have pushed this approach to the discovery of biologically active natural products to the fore [11].

This is obviously not the only way of selecting suitable candidates for investigation. The major emphasis is still on searching for plants in areas of greatest biodiversity but scientists are also looking in more unusual places. Some plants in New Caledonia and Antarctica have been investigated and the deep oceans are currently being searched using submersibles [12]. Fungi, liverworts and cultured chrysophytes have also yielded interesting new compounds over the last few years [13]. Another approach is the reinvestigation of previously poorly studied sources. New separation and structure elucidation techniques coupled with vastly improved bio assay and dereplication procedures turns these species into exciting new prospects. Compounds may not have been present in high enough concentrations for the technology of yesteryear to isolate or may have been in too complex a matrix but today’s scientists have far more powerful tools at their disposal and are able to investigate these “lost” compounds. The largest cost saver in recent years has been the development of various
dereplication techniques – in the form of efficient literature survey methods and in the way bioassays are now conducted to avoid false positive results. It is estimated that for each dereplication approximately US $50000 is saved. This funding can then be used for the isolation and identification of novel bioactive compounds. Testing for biological activity has become far quicker, cheaper and more comprehensive. This means that compounds having undergone bioactivity tests in the past could well possess useful properties that were not detected. Pharmaceutical companies are not as interested in these as they are in new compounds because of the difficulty of obtaining a patent position without a novel structure [2]. Bioassay guided fractionation is now very common and is indeed almost a requirement for funding in the developed countries [14].

A typical investigation (carried out in South Africa) will be used to illustrate the typical approach to the isolation of an active compound from natural sources.
1.2: A Topical Example

*Hypoxis obtusa* is used by Mozambiquan curanderos as a treatment for urinary diseases [15] and it was this lead that prompted Marini Bettolo’s work on the rhizome of the plant as part of his research on African medicinal plants. Hypoxoside (Fig. 1) was isolated and its novel structure was determined. Pegel had already isolated a range of sterols; notably β-sitosterol and its glycosides [16, 17]. A preparation of this extract was originally sold as an agent for the regeneration of the prostate gland and other urinary ailments. The mixture of sterols has been available as an over the counter medicine in Germany for more than twenty years as an immune system booster. A similar product is now available in South Africa under the name “Moducare” which has earned the backing of the MCC. The plant and its relatives, which also contain the active principle [18], are widely distributed bulbous plants in Southern Africa. The Drewes group isolated hypoxoside from *Hypoxis hemerocallidea* (then *H. rooperi*) as part of an investigation into the anti-cancer properties that independent observation had suggested that the plant had [19]. Lay persons had noted that a preparation of the rhizome helped with the healing of malignant sores [20]. The compound showed high toxicity against cancer cells in preliminary tests [21] and is still undergoing testing as an anti-cancer prodrug [22]. This anti-carcinogenic activity was not due to the activity of hypoxoside but rather its aglucone which was named rooperol (Fig. 1) by Drewes (from the old name for the plant) which was formed in the proximity of active cancer cells.
Figure 1: The inactive diglycoside, hypoxoside, is cleaved by $\beta$-glucosidase to form the cytotoxic and lipophilic aglucone, rooperol.

Several reports in the mid-1970s focused attention on high $\beta$-glucuronidase and sulphatase activities in experimental tumours [22]. The idea of designing a prodrug that would be activated by these enzymes at its site of action followed. Nothing in this line was found; bar the mild success of glucuronide conjugates of aniline mustard [23]. Hypoxoside is activated when it loses its two glucose groups in the vicinity of cancerous tumours which produce $\beta$-glucosidase [24]. It then becomes the cytotoxic agent, rooperol and in this way the drug selectively attacks malignant tumours. Rooperol’s mode of action seems to be via an inhibition of leukotriene synthesis [24].

"Up until now, cancer chemotherapy has been a disappointing trade-off between efficacy and toxicity. No chemical agent has yet been discovered that selectively eradicates cancer cells without harming normal cells. One approach to the solving of this intractable problem would be the use of a non-toxic prodrug that is selectively activated to become cytotoxic in the immediate vicinity of cancer cells.”[22]. Hypoxoside is believed to be a step in the right direction in this respect [24]. No toxic effects have been noted in trials with 24 patients and studies “warrant further investigation of hypoxoside as an oral prodrug.”[22]
The initial natural products work stimulated a lot of synthetic research. The Drewes group synthesised multitudes of analogues by various routes (Fig. 2, 3, 4, 5) [25]. Important advances were made in the synthesis of phenolic pent-4-en-1-ynes. A route to obtain phenolic acetylenes in good yield was developed and an improvement in the final deprotection step using Wilkinson’s catalyst was made. Unfortunately none of the analogues possessed the same properties as hypoxoside. All were active but very toxic, active at higher doses, or inactive.
Figure 2: Synthesis of 1,5-bis-(4'-hydroxyphenyl) pent-4-en-1-yn.
Figure 3: Synthesis of 1,5-bis-(3,4-dimethoxyphenyl)-pent-4-en-1-yne
Figure 4: Synthesis of methyl 5-(3,4-dimethoxyphenyl)-2-[(3,4-dimethoxyphenyl)methyldiene]-4-pentynoate

Figure 5: Synthesis of 1,5-bis-(3,4-dimethoxyphenyl)-1-penten-4-one-3-one.
1.2: Natural products work done in Kwa-Zulu Natal in the last five years.

The natural products work done in Natal has been particularly successful with more than one hundred new compounds and over fifty publications appearing in reputable international journals in the last five years from the work of only four main researchers: S.E. Drewes, E. Malan, D.A. Mulholland and K.H. Pegel. What follows is not intended to be a comprehensive review but a collection of relevant highlights.

1.2.1: Drewes

Drewes has continued his program on the isolation and synthesis of compounds from muthi (i.e. medicinal) plants indigenous to Southern Africa. In the last five years his group has worked on a wide range of plants and compounds following leads from various sources. New flavanoids, peltogynoids, α-pyrones, proanthocyanidins and neolignans have been found from a host of different species. Many of these have undergone testing for biological activity. Bioactivity-directed fractionation has also been employed to help increase the 'hit rate' for active new compounds.

*Ocotea bullata* is the most sought after medicinal plant in Kwa-Zulu Natal despite its being declared a specially protected species in 1974 [6]. The substitution by practising herbalists of *Cryptocarya* bark for that of the increasingly rare *Ocotea bullata* bark has been noted on an increasingly large scale [6]. The barks have the same characteristic aromatic smell and both species belong to the family Lauraceae. A search for the chemical link between *Ocotea bullata* and the *Cryptocarya* species to substantiate these observations was therefore of special interest. In this pursuit the Drewes group has isolated eight interesting new compounds from these two genera in the last five years [26,27,28,29,30]. The *Cryptocarya* species have mostly yielded new α-pyrones while the investigation of *Ocotea bullata* has given a new type of neolignan, ocobullenone, and its precursor (see Fig. 6). A possible link was established when ocobullenone and this precursor were isolated from *Cryptocarya liebertiana* [31].
Another tree bark that has yielded interesting new compounds for the Drewes group over the last five years is that of *Cassipourea gerrardii*. The bark of this species is used by Zulu women as a face pack and is sold extensively in herbal and medicinal plant markets as a skin lightener [32]. Earlier work done by Warren and his associates showed the presence of sulphur containing alkaloids [33]. Two *Cassipourea* species have been investigated and have given new flavanols, flavanol glycosides, proanthocyanidins and doubly linked dimeric proanthocyanidins (Fig 6) which may be the active compounds in the bleaching process [34].

*Cassine transvaalensis* was first investigated because of its close relation to other members of the family that had yielded quinonoid triterpenes with anti-tumour activity [35]. The tree’s bark gave a new and rare type of peltogynoid (Fig 6) and a comparative study was started on *Cassine papillosa*. This tree showed much the same composition [36].

*Bulbine latifolia* is used in Southern Africa for the treatment of diarrhoea, dysentery and rheumatism. Knipholone (Fig 6) was isolated from this indigenous bulbous plant for only the second time outside the genus *Kniphofia*. The same compound was also found in *B. frutescens* [37,38]. The presence of knipholone holds taxonomic importance when coupled with previous findings by Van Oudtshoorn who reported the presence of crysophanol in *Bulbine* [39]. The common occurrence of these two compounds indicates a close relationship between the genera *Bulbine* and *Kniphofia* and may support the placement of *Bulbine* in the subfamily Asphodeloideae. In view of previous reports on biological activity in chiral biaryls microbial tests were undertaken with no positive results.
(+) Afzelechin-3-rhamnoside from *Cassipourea gerardii*.

(+) 11, 11-Dimethyl-1,3,8,10-tetrahydroxy-9-methoxypeltogynan from *Cassine transvaalensis*.

Ocobullenone from *Ocotea bullata*.

Epiafzelechin-(4β =>8, 2β =>0 =>7)-ent-afzelechin from *Cassipourea gerardii*.

Cryptofolione from *Cryptocarya latifolia*.

7-Styryl-2, 6-dioxabicyclo [3,3,1] nonan-3-one from *Cryptocarya myrtifolia*.

Figure 6: Some of the new compounds isolated by Drewes over the last five years.
In continuation of a project, started by Taylor in the 1970’s, Mulholland has isolated many new limonoids and protolimonoids from the family Meliaceae over the last five years. Structurally, the limonoids are derived from tetracyclic triterpenes by the formation of a β-substituted furan group on ring D with the loss of four carbon atoms. This is achieved by a series of oxidative changes, interspersed with molecular rearrangements. Many limonoids have been shown to be potent insect anti-feedants [40] and it is probable that this is the biological advantage that these compounds convey to members of the Meliaceae. Limonoids of the havanensin and prieurianin groups, which contain 14,15 epoxide rings, have been shown to be active against certain types of cancer [41]. Mulholland has not, however, limited herself to this class of compounds and has discovered new triterpenoids and dammaranes from the Meliaceae. Increasingly sophisticated techniques have been employed and many of the compounds have been tested for biological activity. Supercritical fluid extraction and micellar electrokinetic capillary chromatography are some of the methods used to enhance separation of these sometimes intractable compounds [42,43,44] which are often very similar, frequently unstable and may interconvert on thin layer chromatography.

Mulholland has investigated members of the Meliaceae from Africa, Madagascar and Australia in the last five years. The African plants gave new limonoids and triterterpenoid acids [45,46,47,48,49,50]. A taxonomic debate involving the placement of Nymania in the tribe Turraeae was solved by the confirmation of a chemotaxonomic link between Nymania and Turraea [46]. Madagascan members yielded novel limonoids and dammaranes. Dammaranes are often found in conjunction with limonoids in the Meliaceae but no structural relationship or taxonomical significance has been established as yet [50]. The isolation of asterotrichilin (Fig 7) suggests a relationship between Asterotrichilia and Ekebergia [51]. Novel coumarins were isolated from Ekebergia sp. [55]. The Australian Meliaceae were thoroughly investigated with the omission of only two genera [52,53,54,55]. The first and, to date, only isoflavanoid to be isolated from any
member of the family was one of the compounds isolated [56,57]. Other new compounds were limonoids and triterpenoids. A taxonomic advance was the confirmation of the separation of the genus *Xylocarpus* into three separate species by chemotaxonomic means.
Figure 7: Some of the new compounds isolated by Mulholland over the past five years.
Malan specialises in the isolation of phenolics from indigenous Acacia species but he has also worked on other plants and compounds in the last five years. He isolated the first proteracacinidin dimer with a double linkage among many other new proteracacinidins and flavanoids. He has also cleared up some pertinent taxonomic debates with respect to the most widespread Acacia species in southern Africa. In a joint project with Ferreira, from the University of the Orange Free State, biomimetic syntheses have been effected on many of these new compounds. Fungicidal and growth inhibition tests have been carried out on many of the new compounds isolated. New compounds have also been isolated from Combretum and Distemonanthus species.

*Acacia karroo* is the most widespread acacia species in southern Africa [58]. In the past botanists have proposed that *A. karroo* be subdivided into several subspecies but met with resistance from the Botanical Society of South Africa [59]. Malan has investigated the chemical constituents of the heartwood of specimens of this species collected from two locations. The metabolic pool present in the two was shown to differ considerably with the proposed subspecies *montana* containing a greater variety of 7,8,3',4'-substituted flavonoids and a large number of O-methylated derivatives. No O-methylated flavonoids were found in *A. karroo* while four new O-methylated flavonoids were found in the subspecies *montana* [59].

In 1994 Malan isolated the first two proteracacinidin dimers from *Acacia galpinii* confirming their structure by biomimetic synthesis (Fig 8) [60,61]. The predominating proteracacinidin in the heartwood, epioritin-4α-ol, was used as a starting material and both dimers were formed in good yield under mild acidic conditions. Later in the same year another biflavonoid of the same type but with a unique 4β→7, 5→6 double linkage was isolated from *Acacia caffra*. The 4β→7 ether linkage had never been observed before. Other new dimers and several new flavonoids were discovered in the genus *Acacia* [62, 63,64].

*Distemonanthus benthamianus* heartwood has produced a new flavonolignan with a
rare 1,2,3,4-tetrasubstituted D-ring (Fig 8) \cite{65}. Benzodioxane lignoids have been claimed to possess medicinal properties \cite{66}. The compound is the first benzodioxane-linked flavonolignan to be discovered. A phenylpropane unit is attached to a flavonol moiety in a novel way in this compound. *Cassine abbreviata* was also investigated and yielded many known compounds and two previously unreported proguibourtinidol oligomers. Many of these structures were confirmed by synthesis using condensation under mild acidic conditions \cite{67}. Substituted bibenzyls, phenanthrenes and 9,10-dihydrophenanthrenes were isolated from the heartwood of *Combretum apiculatum*. Growth inhibition and fungitoxic screening tests were conducted on the phenanthrene type compounds \cite{68}. 
Ent-orientin-(4β→5) epioritin-4β-ol from *Acacia caffra*.

(2S,3R-3,10-dihydroxy-9-O-(6′hydroxy-7′-O-methyl-2′-hydroxydihydrobenzo furan-3-yl) dibenz-[b,d]-pyran-6-one from *Acacia karroo*.

2,3-trans-2-(4-hydroxy-2,3-dimethoxyphenyl)-9-(5-hydroxy-2-methoxyphenyl)-hydroxymethyl-2,3-dihydro-7H-1,4-dioxino[2,3-h]chromen-7-one from *Distemonanthus benthamianus*.

Ent-orientin-(4β→7,5→6) epioritin-4α-ol from *Acacia caffra*.

*Figure 8: Some of the new compounds isolated by Malan over the last five years.*
Pegel submitted three papers with Taylor and Ansell that were received by Phytochemistry on the same day, describing thirty-seven new diterpenes, along with 66 known ones, from various Erythroxylum species. In this thorough investigation a new class of diterpene was found and named.

The genus *Erythroxylum* incorporates about 200 species that are chiefly found in the tropical regions. It is best known as the source of the alkaloid cocaine. The genus has also been shown to be a source of a wide variety of diterpenes. Twenty-two species of *Erythroxylum* from around the world were investigated with *E. pictum* and *E. australe* proving to be most fruitful in the search for new compounds. *Erythroxylum pictum* is an indigenous tree that is common in Natal Coastal forests. The tree was found to contain a group of 4,5-seco- rosanes that were given the name pictanes (Fig 9) [69]. Erythroxydiol Y was also isolated and the name dolabrane was proposed to describe other molecules with the same 8,14-friedo-pimarane skeleton (Fig. 9). In all; six new pictanes, four new labdanes and two new rosanes were discovered.

*Erythroxylum australe* was originally examined in order to obtain reference compounds for the ongoing work on *E. pictum*. The tree had been investigated before and found to contain diterpenes in the roots [70]. Ten new diterpenes were isolated and identified along with twenty-one previously reported compounds. The new compounds were all of the beyerane skeleton (Fig. 9). The name devaderane was proposed for compounds related to erythroxydiol X.

Twenty other Erythroxylum species were investigated, thirteen of which were found to contain diterpenes [71]. The twelve new compounds identified in this study were of the rosane, pimarane, dolabrane and beyerane types (Fig 9).
ent-17-hydroxybevry-15-en-1-one from *Erythroxylum australie* showing the beverane skeleton.

ent-11β-acetoxy-5c6ros-1(10)-en-15E,16-diol from *Erythroxylum zambeziacum*, an example of a rosane.

ent-15E,16-dihydroxypictan-4(18)-en-5-one from *Erythroxylum pictum* exhibiting the pictane skeleton.

ent-15E,16-dihydroxydolabr-4(18)-en-1-one from *Erythroxylum pictum*, an example of a dolabrane.

Erythroxydiol Y.

ent-Devaderan-1β,11β,15E,16-tetrol.

Figure 9: Some of the compounds isolated by Pegel over the last five years.
Chapter 2

Discussion.

2.1: Ocotea kenyensis

The Lauraceae were probably named for the laurel wreaths of Roman times which were made from Bay (*Lauris nobilis*) leaves, but the family encompasses a wide variety of different trees found in tropical and sub-tropical climates around the world [72]. Commercially important species like Avocado, Cinnamon, Sassafras and Sweet Bay have been placed in this family [5, 72]. Perfume oils have been obtained from *Aniba duckei* (rosewood), *Aniba rosaoedora* and *Ocotea pretiosa*. Ethnopharmaceuticals come from *Aniba coto* and *Ocotea rodiae* i in the form of coto bark and demerara-greenheart respectively [72].

The indigenous Southern African Lauraceae are composed of the *Cryptocarya*, the *Ocotea* and the *Dahlgrenodendron* genera. The best known species is the Black Stinkwood, *Ocotea bullata*, which is now rare for three main reasons. The massive reduction in the area covered by indigenous forest was the largest contributing factor followed by the exploitation of the species by the timber industry. This is now largely under control since the tree was declared a specially protected species in 1974 [6]. The greatest threat to the existing specimens is the muthi trade. *Ocotea bullata* was the most sought after muthi plant in Kwa-Zulu Natal in a survey done by the Institute of Natural Resources. The tree is increasingly difficult to find and specimens are, more often than not, ring barked by gatherers when located. Substitution of *Cryptocarya myrtifolia* has been noted [6, 22] and this was the subject of previous research in these laboratories where tentative chemical links were established between the two genera [22].
It was of interest to investigate the phytochemical relationship between *O. bullata* and *O. kenyensis* particularly since very little is known of the chemistry of *O. kenyensis*. In addition, this investigation offered the possibility of comparing the chemical composition of the leaves and the bark. This relationship has commercial interest [73] since there are inherent advantages in harvesting leaves rather than bark. Trees would not be damaged irreparably by the barking procedure and leaves could be harvested sustainably. This is an aspect currently under investigation by the Institute of Natural Resources.

Many new compounds have been isolated from *Ocotea bullata* by researchers in these laboratories over the past five years [18, 28]. Among these were ocobullenone (2), iso-ocobullenone (I) and their precursors. The presence of these types of compounds in *O. kenyensis* was expected from the results of comparative tlc plates and was confirmed with much difficulty. This was due to the presence of many other compounds with similar R_f values as can be seen from the diagrammatic tlc plate (Fig 10) comparing the extracts of *O. kenyensis* and *O. bullata*. This study has shown that *O. kenyensis* contains iso-ocobullenone and its precursor in both the bark and the leaves in roughly the same percentage of the dry weight of plant material. It is important to note that where ocobullenone had been the major isomer in *O. bullata* its iso form was more prevalent in *O. kenyensis*.
Compound 7 and at least two other compounds

Compound 6

Purple spot

Compounds 1, 2, 3, 4 and other compounds

Green spot

Compound 21

Compound 5

*Ocotea kenyensis*  Ocobullenone  *Ocotea bullata*

Figure 10
Iso-ocobullenone (1) was isolated after repeated column chromatography followed by preparative layer radial chromatography. GCMS showed the [M]⁺ ion to be 370 mass units and the molecular formula of C₂₁H₂₂O₆ was confirmed by high resolution EIMS. Iso-ocobullenone differs from ocobullenone only in the configuration at C-8 and this was proved previously by X-ray crystallography [18, 28]. It was the proton NMR signal of the methyl group attached to this carbon that was used to discern between the two isomers, both of which were present in the leaves and bark of *O. kenyensis*. In ocobullenone, the C-9 methyl group lies in the same plane as the benzene and the cyclohexanone rings, which is above the general plane of the cyclopentane ring. In iso-ocobullenone the methyl group is below the plane of the cyclopentane ring. The doublet arising from the protons on C-9 was shifted accordingly, 1.16 ppm in iso-ocobullenone vs. 0.89 ppm in ocobullenone (Fig 11). This was a useful indicator in the determination of the degree of separation after each chromatotron run.
Figure 11
Figure 12
2.1.2: $\Delta^8$-3,4,5-trimethoxy-3’, 6’-dihydro-3’,4’-methylenedioxy-6’-oxo-8,3’-neolignan

The isolation of $\Delta^8$-3,4,5-trimethoxy-3’, 6’-dihydro-3’,4’-methylenedioxy-6’-oxo-8,3’-neolignan (3), a possible precursor to iso-ocobullenone, proceeded slightly more smoothly than the isolation of iso-ocobullenone and it was isolated in identifiable form after three successive columns and one chromatotron run. Identification of the compound was achieved primarily by comparison of $^{13}$C NMR data with literature values [74] and this was confirmed by high resolution EIMS which suggested a $\text{C}_{22}\text{H}_{28}\text{O}_6$ compound. The compound is almost certainly a mixture of two isomers with different stereochemistry at C-8 and possibly at one of the other chiral centers. This can be seen in the $^1$H NMR spectrum where two doublets arising from the protons on the C-9 methyl group are clearly separated (Fig 13 0.86 and 1.02 ppm) but with the appearance of a third doublet at 1.14 ppm, indicating that a third chemical environment exists in the mixture. The presence of a majority of the iso-ocobullenone precursor is indicative of the ratio of iso-ocobullenone to ocobullenone in $O$. *kenyensis.*
2.1.3: New compounds related to iso-ocobullenone

Two related compounds were isolated from the leaves of *O. kenyensis* in very small quantities (1-5 mg). This prevented the repeated application of chromatographic techniques that allowed the isolation of iso-ocobullenone and bullatone. This, together with the complexity of the extract, resulted in mixtures of compounds being isolated. Proton NMR and high-resolution mass spectra verified their similarity to the previously isolated compounds. The compounds had molecular formulas of C$_{21}$H$_{24}$O$_{5}$ and C$_{23}$H$_{30}$O$_{6}$ respectively as determined by high resolution EIMS. Their proton NMR spectra showed the presence of very similar arrangements of atoms but with differing oxidation patterns on ring A. Possible structures for these two compounds are shown below (4, 5).

![Diagram](image)

The lower mass compound (4) was isolated with fewer contaminants and an assignment of $^1$H and $^{13}$C NMR peaks was possible. The methyl group attached to C-8 is definitely of the iso configuration due to its shift on $^{13}$C NMR (17.0 ppm) which is higher than that of ocobullenone and its precursor, 14.2 and 13.3 ppm respectively. Indeed the shift matches that of iso-ocobullenone almost exactly (17.0 vs. 16.9 ppm) (Fig. 16). The shifts of carbons 7 and 1' show that the compound is certainly linked to form the cyclopentane ring as in iso and ocobullenone. It remains only to place the two methoxy groups on ring A. This was fairly obvious by the equivalence of carbons...
5 and 3 and of carbons 6 and 2. This suggested two meta methoxy groups. The remaining carbons are very similar in their $^{13}$C NMR shift values to iso-ocobullenone. Two-dimensional spectra could not be run with only 3mg of sample and a 200MHz instrument. The structure proposed for this compound has not been previously reported.

The higher mass compound, 5, was isolated as an approximately equal mixture of two isomers differing in their configuration at the same chiral center, C-8. The extra methoxy peaks can be seen around 3.8 ppm in the proton spectrum but the presence of contaminants makes identification of this compound more difficult.

The similarity between the constituents of the bark and leaves is shown by the presence of iso-ocobullenone and its precursor in both sources in approximately the same percentage of the dry mass. The presence of two related compounds in the leaves supports this hypothesis.
Two isoprenoid type compounds were isolated from the leaves of *O. kenyensis*. *E,E,Z,Z,Z,Z,Z,Z,Z-Z-Undecaprenol (6)* is a constituent of many leaf waxes [75]. In the identification of this compound the $^1$H NMR showed immediately that an isoprenoid had been isolated and only the doublet at 4.09 ppm and the triplet at 5.44 ppm remained as obvious pointers toward a terminal hydroxy group (See Chapter 4). The signals arising from the protons of the methyl groups that are in the E configuration are shifted slightly upfield to 1.60 ppm as opposed to the Z methyl groups which appear at 1.68 ppm. These integrals allowed the assignment of the number of E vs. Z double bonds but not their position. Comparison of the carbon and proton NMR data with literature values [75] showed an exact correlation with those of *E,E,Z,Z,Z,Z,Z,Z,Z-Z-undecaprenol*, or bacterialprenol, and this structure is therefore proposed.
The proton NMR of the second isoprenoid (7) isolated from *O. kenyensis* suggested that it was uniformly of the *Z* configuration and this was supported by the shifts of the methyl groups in the $^{13}$C NMR spectrum where only one trans methyl signal appears at 25.7 ppm. This is the terminal methyl group. The COSY spectrum showed that the doublet of triplets at 3.11 ppm was linked to the triplet at 6.48 ppm (Fig 17) and it was mostly the shifts and splitting pattern of these two signals that spawned the hypothesis that a terminal $\alpha,\beta$ unsaturated ketone was present. This was supported by shifts on $^{13}$C NMR with the exception of carbon 2 which was, presumably, lost in the noise and omitted from the spectrum (See Chapter 4). Mass spectral data was not obtainable due to the fact that the compound was not stable to GC/EIMS as in the case of the previous compound and squalene. The mass of both compounds was inferred by the integrals on proton NMR. The proposed structure has not been previously reported.
2.1.5: Other constituents.

Other known compounds isolated from *O. kenyensis* were β-sitosterol and triacontanol. The spectral data of these two compounds matched those of the literature [76, 105].
2.2: Apodytes dimidiata

The Icacinaceae, or white pear family, is a tropical and sub-tropical family with three tree species indigenous to Southern Africa [5]. One of these is *Apodytes dimidiata*, which has been a subject of taxonomic debate in the past. There has been uncertainty as to the number of species contained in the genus [77]. Between 2 and 17 species are recognized depending on the authority. The trend in recent years has been to view the genus as one widespread and polymorphic species, *Apodytes dimidiata*. The fruit of the species is inedible but the wood is hard, strong and elastic and was used in wagon construction. The Zulu people use an infusion of the bark mixed with other plants as an enema for intestinal parasites. The leaves of the tree are used by the Luo people to treat ear inflammation [78]. A full description of the genus is to be found in Kayonga’s thesis [87].

Previous studies on the plant have yielded new diterpenes and diterpene based alkaloids [79] of the type shown below (Fig 18). Genipin (8) and betulinic acid (12) were isolated from *A. dimidiata* in a previous study in these laboratories [80].

![Icaceine](image1.png)

![Icacinol](image2.png)

*Figure 18*
2.2.1: Molluscicidal potential

It has been reported that the weakest link in the life cycle of the schistosome that causes bilharzia is the intermediate snail host [81, 82]. Destroying the snails which harbour the developing schistosome larvae is the one way to interrupt the parasite’s life cycle and prevent human infection that is potentially affordable for the poor countries that are affected by the disease [83]. The World Health Organization estimated in 1989 that about 200 million people had contracted schistosomiasis and that a further 600 million were at risk of infection [84]. Nearly all the people at risk are in Third World countries where slow moving or stagnant water is used as a source of drinking water or for washing [85].

Niclosamide (9)
Niclosamide (9) is the only commercially available product and the high cost of this compound, along with the expenses required to apply it, have prevented its use in all but the most severe situations. The chemical also has long term effects on the environment [86]. The need for a cheap and effective molluscicide is obvious.

Many plant extracts have been examined in the search for new molluscicides and a vast range of compounds has been found to be active with varying degrees of efficiency [83]. The most outstanding work is that done by Dr Akilulu Lemma in Ethiopia on *Phytolacca dodecandra*. The triterpenoid saponins isolated from the berries of this species have been shown to have LD$_{100}$ values as low as 2 ppm against *Bulinus africanus* [83]. The plant has subsequently become of great potential importance for the local control of schistosomiasis [83].

*Apodytes dimidiata* was selected from a large collection of indigenous plants as a possible molluscicide in a study carried out by Clark [80]. By employing a scoring system based on the criteria for “good” molluscicides as defined by the WHO the field was narrowed down from a large number of potential candidates to only three. These were *Apodytes dimidiata*, *Gardenia thunbergia* and *Warburgia salutaris*. A previous study on *Apodytes dimidiata* [87] yielded positive results. Isolation was guided by the activity of different fractions and this lead to the identification of genipin (8) in *Apodytes dimidiata* as a potentially useful molluscicidal agent [80] and subsequent tests on the pure compound reveal that it is indeed a potent molluscicide. Using *Bulinus africanus*, the intermediate host of *Schistosoma haematobium*, as the target organism, an LD$_{50}$ value of 25.27 ppm (±1.77 ppm) and an LD$_{90}$ value of 39.40 (±14.85 ppm) was established. It has since been revealed that toxicity effects of *A. dimidiata* extracts on earthworms, fish and other small mammals (rabbits) are extremely low [88]. This would be a prerequisite for any commercially viable molluscicide.
2.2.2: Triterpenoids.

Squalene.

Squalene (10) was isolated as a clear oil from the dichloromethane extract and was identified as an isoprenoid from its proton NMR spectrum which showed characteristic signals at 1.60, 1.67, 2.17 and 5.10 ppm. The ratio of trans to cis methyl groups could be seen from the integrals of the singlets at 1.60 and 1.67 and it was apparent from this that only the terminal methyl groups were trans leaving the remaining units uniformly of Z configuration. The presence of only “one” terminal trans methyl group indicated that the molecule was symmetrical. The $^{13}$C NMR data, along with the COSY and HETCOR spectra, supported the idea of a chain of Z isoprene units and confirmation of the structure was by comparison of $^{13}$C NMR data with those of the literature [89]. Mass spectral data were not available because the compound fragmented at the temperatures needed to elute it from the GC column being used.
The isolation and identification of lupeol (11) from the dichloromethane extract of A. *dimidiata* proceeded smoothly with only a single column separation and a chromatotron run required. The proton NMR showed the typical triterpenoid form and the presence of a terminal double bond with the signals at 4.57 and 4.68 ppm as well as a proton attached to a carbon with a hydroxyl group (3.19 ppm). The $^{13}$C NMR data was compared to literature values and a good correlation was found [90].
The presence of betulinic acid (12) was suspected from the results of an examination of initial tlc plates. It was found to be present in large quantities (ca. 0.3 % dry mass of leaves). The compound precipitated en masse upon concentration of the polar fractions of a short column run with a solvent gradient. Solubility proved to be the only problem in the identification of the compound and deuterated pyridine was used as the solvent for NMR. The compound had been isolated from the same plant by researchers in these laboratories in a previous study [87] and thus comparison of $^1$H and $^{13}$C NMR data facilitated characterization. The same two peaks that showed the presence of a terminal double bond in lupeol were apparent as well as the proton signal arising from the hydrogen on carbon 3.
2.2.3: Other constituents.

Catechin.

(C13)

Catechin (C13) was identified primarily from its proton NMR spectrum that was characteristic of the flavan-3-ol type and was compared with spectra of catechin isolated previously in these laboratories. The sample was slightly contaminated with genipin as can be seen from the singlet at 3.71 ppm in the proton NMR spectrum representing the methyl ester protons of this compound. The sample was run in deuterated methanol, which lead to a grossly exaggerated hydroxyl peak at 4.90 ppm. The assignment of the stereochemistry at C-2, which differentiates between catechin and epicatechin, was achieved by comparison of the spectral data (both $^1$H and $^{13}$C NMR) with those quoted in the literature [91].

Benzoic acid and sucrose were also isolated.
2.2.4: Synthesis of 4-Ethyl catechol.

The synthesis of 4-ethyl catechol (14) was undertaken in order to confirm the structure and molluscicidal activity of a compound isolated from *Gardenia thunbergia*, one of the species which showed promise in Clark’s study [80] as discussed earlier. The compound, labeled GT1, was isolated in these laboratories in very low yields [87]. Since it contained some contaminants it was deemed necessary to show that it was the 4-ethyl catechol and not the other compounds present that accounted for the toxicity shown in tests conducted in 1995 (Table 1).

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>% Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td>90</td>
</tr>
<tr>
<td>10</td>
<td>85</td>
</tr>
</tbody>
</table>

LD$_{50}$ = 4.80 ppm (2.48 – 9.29)
LD$_{90}$ = 9.48 ppm (4.90 – 18.35)

*Table 1*

Several synthetic routes were considered. A reference in the patent literature suggested that caffeic acid could be decarboxylated by refluxing in DMF (24 hrs) [92]. No change was noted on tlc after four days reflux and this method was abandoned. Next a synthesis based on 3,4-dihydroxy benzaldehyde leading via piperonal and saffrole to the target molecule was then investigated (Fig 19).
First it was necessary to protect the two phenolic groups on 3,4-dihydroxybenzaldehyde since they would interfere with the proposed Wittig coupling. In this case the lack of an electron-withdrawing group to stabilise the methyltriphenylphosphonium ylide makes it far more reactive than stabilised triphenylphosphonium ylides. Initially protection using more easily removable groups than the methylenedioxy moiety was attempted, but the use of any base caused polymerisation of 3,4-dihydroxybenzaldehyde and a correspondingly lower yield that was unacceptable in the first step of the synthesis. Benzylation of the two phenolic groups on 3,4-dihydroxy benzaldehyde using potassium carbonate as a base gave the anticipated dibenzyloxy-protected compound, but in addition gave a compound in which benzylation had occurred at C-2 (15) even when exactly two equivalents of
benzylchloride were used. This presumably arises from a Friedel-Crafts like alkylation reaction. The $^1$H and $^{13}$C NMR spectra are shown in Figures 21 and 22.

$$\text{(15)}$$
The first step of the synthesis involved the formation of the methylenedioxy protecting group using diiodomethane. This resulted in the formation of piperonal in high yield (99%).

The Wittig reaction was then used to effect the conversion of the aldehyde to a terminal double bond and saffrole was formed in high yield (79%) with little effort [93]. The reaction was effected under an atmosphere of nitrogen and DMSO was used as a solvent to allow a shorter reaction time.

The hydrogenation of the terminal double bond was achieved using palladium on activated carbon as a catalyst. The absorption of hydrogen gas was monitored to assess the degree of completion of the reaction.

The removal of the methylenedioxy protecting group was effected using a method described by Williard and Fryhle [94] after conventional procedures proved to be ineffective. These authors report high yields for the dealkylation of aryl ethers using boron trihalide-methyl sulfide complexes. The authors suggest that an equilibration occurs between the methyl sulfide complex and the aryl ether which compete as Lewis bases for the coordinately unsaturated boron species [94]. This equilibrium allows a mild in situ release of the boron trihalide species as a powerful Lewis base.

The end product was shown to be the desired target molecule by the usual spectroscopic procedures.
2.2.5: Testing

The synthetic product was tested using the same technique as that used in the study done in 1995 [87]. The results of the tests are shown in Table 2.

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>% Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>35</td>
</tr>
<tr>
<td>9</td>
<td>50</td>
</tr>
<tr>
<td>12</td>
<td>60</td>
</tr>
</tbody>
</table>

$LD_{50} = 27.58 \text{ ppm (25.34 – 30.04 ppm)}$ 95% CI  
$LD_{90} = 36.58 \text{ ppm (31.57 – 42.54 ppm)}$ 95% CI

Table 2

The increase in the $LD_{50}$ and $LD_{90}$ values can be attributed to the use of an alternative water source. The previous study was conducted using chlorinated tap water or distilled water whereas in this study stream water was used. It can be assumed that chlorinated, de-ionized and distilled water would increase mortality in the snails, as their osmotic balance would be upset. The osmotic potential of these waters is very high and the snails in this environment would be under stress as they tried to prevent over-hydration. It is for this reason that water from the snails’ natural habitat was used. The risk of introducing other factors that might affect the toxicity of 4-ethyl catechol was considered but this was outweighed by the fact that these would be present in the situation in which the product might eventually find use. The reduced toxicity may in fact be due to adsorption of the chemical onto organic matter in the water.

It is, however, certain (by comparison of spectra) that the structure of the compound isolated by Kayonga in the previous study was indeed that of 4-ethyl catechol. The test results also show that it was this compound that was responsible for the molluscicidal activity seen in the 1995 tests as the tests done in tap water gave very similar results.
4-Methyl catechol was available and was tested as an analogue of the ethyl compound. It was shown that this compound was in fact slightly more effective as a molluscicide than the original compound. The LD$_{50}$ was 22.99 ppm (20.75 – 25.45 ppm) and the LD$_{90}$ value was 30.49 ppm (25.48 – 36.45 ppm).

These toxicity results are reasonable but not low enough to be of major commercial value.
2.3: **Ehretia rigida**

The Borage or Heliotrope family is large and cosmopolitan and is best known for its garden flowers and herbs that include forget-me-nots, heliotropes, pansies and the herbs comfrey and burdock [5]. The family has yielded many interesting compounds like delphinidin, cyanidin, malvidin, quercetin and kaempferol [95]. Two genera grow as trees in Southern Africa, *Cordia* and *Ehretia*. Figure 24 below shows some of the compounds that have been isolated from these genera.

- **Artemetin**: An anti-inflammatory agent with low toxicity from *C. verbenacea*
- **Lasidiol angelate**: An ant repellent from *Cordia* sp.
- **Cordiachrome A**: A potentially useful dye from *Cordia chacoensis*.
- **Ehretinine**: An interesting pyrolizidine alkaloid from *Ehretia* sp.

**Figure 20**

*Ehretia rigida* is also known as the puzzle bush and is a shrub or small deciduous tree that grows to between 2 and 8 meters. It is common and widespread. The fruit is
edible and the stems are used by the Zulu people for medicinal and magical purposes as well as to make fishing baskets and spear shafts [5, 78].

Very little is known about the chemistry of E. rigida and no publications were found that dealt with the phytochemistry of this species. The tree was selected on the basis of discussions held with researchers in Kampala [96] that suggested that the species might contain trypanocidal compounds. The tree was investigated in the hopes of finding a compound that showed trypanocidal activity at low concentrations and that did not exhibit toxicity against mammals.

Trypanosomiasis (also called Chaga's disease) is caused by the flagellate protozoan Trypanosoma cruzi. The disease is transmitted in nature by blood sucking triatomine bugs [97]. It has a multistage life cycle with different developmental stages in different hosts [98]. In its acute phase, trypanosomiasis is seldom fatal in humans and resolves spontaneously in a few weeks. The patients, however, do remain parasitemic and insects and blood transfusion transmit the disease. Many years after acquiring the disease up to 30% of patients develop symptomatic disease that usually affects the heart, leading to death, or less frequently the gut, causing megaesophagus or megacolon. Between 16 and 18 million people are estimated to be infected with T. cruzi from Mexico to central Chile and southern Argentina alone [99].

The only drugs used in the early stages of trypanosomiasis, which usually go undetected, are nifurtimox and benznidazole. Toxic reactions occur in about 50% of the patients treated with these drugs and they are both ineffective in treatment of the advanced disease [100]. The need for development of improved drugs for the treatment of this prevalent disease is obvious.

Bioassay guided fractionation was used to focus the isolation process on active extracts. The crude extracts were tested and the chloroform and methanol extracts were shown to be the most active. Neither of these results was exceptionally high and the project was not pursued further.
2.3.1: Allantoin

One of the major components of the bark was found to be allantoin (16) (ca. 0.1% of the dry mass of bark). This compound produced interesting spectral data in that very few signals were observed on both $^1$H and $^{13}$C NMR. Mass spectral data was unavailable until the direct probe technique was used and even this gave seemingly anomalous results when the number of carbons shown in the $^{13}$C NMR spectrum was taken into account. It was the singlet at 10.54 ppm in the proton NMR spectrum that was used as a guide to the structure of the molecule before the results of the X-ray crystallography experiments were known. The singlet, arising from the proton on the nitrogen in the six position, was used in a previous study to determine the amounts of allantoin present in crude extracts [101]. The signal was shifted further than the protons of other compounds in the extracts and a quantitative measurement of the amount of allantoin in the extract was obtainable from the integral values. This lead to the comparison of spectra with literature data and good correlation was shown. Further confirmation was supplied by the results of the crystallography experiments (Chapter 5).

Allantoin is a product of purine metabolism in a wide range of organisms [102]. It is formed from uric acid with the loss of CO$_2$ by the action of the enzyme uricase. Nonprimate mammals, gastropod molluscs and some insects excrete allantoin. The compound is used in living organisms to hold nitrogenous waste in a non-toxic form until it can be excreted and this is almost certainly the reason for its presence in *Ehretia rigida*. 

![Allantoin Structure](image)
2.3.2: α and β-Amyrin.

A mixture of α and β-amyrin (17 and 18) was isolated in large quantities from both the hexane (10 g) and the chloroform (3 g) extracts. A third compound was shown to be present on GC analysis and this is suspected, from spectral data, to be 7-baueren-3-ol. These three compounds have been isolated from Cordia and Ehretia species before [103]. The three compounds co-crystallized and were inseparable due to their similarity of structure. Chromatotron runs did allow the isolation of mixtures that contained a majority of each of the compounds but the total exclusion of the remaining two compounds was not achieved. Acetylation and benzylolation had no effect on separability. Emphasis was not placed on these compounds once it was clear that the structures were not novel and the mixture showed low toxicity in the bioassay.

2.3.3: Other compounds.

1-Triacontanol was isolated in crystalline form from the chloroform extract and spectral data showed good correlation to that in the literature [104]. The bark also gave β-sitosterol that was also identified by comparison with spectral data in the literature [105].
2.4: Conclusion

The isolation of iso-ocobullenone (1) and its precursor (3) in the leaves of Ocotea kenyensis is significant because the presence of the same chemicals in the leaves and bark allows substitution of one for the other in the muthi trade. The ring barking of the closely related Ocotea bullata is the greatest threat to this increasingly rare species. Leaves are far more sustainably harvestable and their replacement for bark would contribute to the preservation of Ocotea bullata. The leaves of Ocotea kenyensis have yielded compounds related in structure to iso-ocobullenone. One of these, Δ⁸⁻3,5-dimethoxy-3',4'-methylenedioxy-1',2',3',4'-tetrahydro-6'-oxo-7,1',8,3'-neolignan (4), is described here for the first time. Other compounds isolated were bacterialprenol (6), β-sitosterol (21), triacontane (22) and a new isoprenoid (7).

The investigation of Apodytes dimidiata as a potential source of molluscicides for the control of bilharzia transmitting snails was continued. The known compounds squalene (10), catechin (13), lupeol (11) and betulinic acid (12) were isolated. These were not deemed to be of value in this respect. A previously isolated compound, 4-ethyl catechol (14), was synthesized in order to confirm its structure and molluscicidal activity. The toxicity results obtained from the tests conducted were reasonable but not high enough to be of commercial value.

Ehretia rigida was investigated in a bio-assay guided fractionation project to determine the presence of compounds exhibiting toxicity against schistosomes that cause sleeping sickness. Allantoin (16), α- and β-amyrin (17, 18), triacontanol (23) and β-sitosterol (21) were isolated. The activity of the extracts was not high enough to warrant detailed investigation.
Chapter 3

Experimental

3.1: Instrumentation

Melting points were measured on a Kofler hot-stage apparatus and are uncorrected. Elemental analyses were determined using a Perkin-Elmer 2400 elemental analyzer. NMR spectra (\(^1\)H 200MHz and \(^{13}\)C 50 MHz) were recorded on a Varian Gemini 200 instrument at room temperature. The solvent used, unless otherwise specified, was deuterated chloroform with TMS as the internal standard. Mass spectra were recorded on a Hewlett-Packard gas chromatographic mass spectrometer (HP5988A) and for high resolution mass spectrometry a Kratos MS 80 RF double-focussing magnetic sector instrument was used. Optical rotations were determined on a Perkin-Elmer 241 polarimeter. Infra-red spectra were recorded on a Shimadzu FTIR-4300 spectrophotometer using KBr discs unless otherwise stated.

3.2: Chemicals

Precoated Kieselgel 60 F-254 Merck plastic sheets were used for thin layer chromatography. Preparative column chromatography was performed using the technique of Still et al. [106] on Merck silica gel 60 (230-400 mesh). Anisaldehyde dip reagent was used in the development of plates unless specified to the contrary. Solvents were dried using standard techniques [107] and distilled prior to use.
3.3: Extraction of plant material.

Plant material was air dried for at least seven days prior to extraction. Bark samples were then milled while leaves were crushed using a mortar and pestle. Unless specified to the contrary plant material was then extracted with hexane, chloroform, ethyl acetate and methanol successively by standing it in the solvent for two weeks at room temperature. Unless otherwise stated the plant material was filtered off and the solvent removed \textit{in vacuo} until a gum or paste was obtained for weighing. The mass was ascertained and the extract was loaded onto a plug of silica gel (±5 cm) in a suitable column. Large fractions (about 20ml) were collected unless obvious bands were visible and amenable to separation and a solvent gradient was applied to ensure that the vast majority of the material was recovered. The fractions were then examined on TLC and combined according to their similarity. The solvent was removed and the new fractions were subjected to flash column chromatography using the technique of Still \textit{et al.} [106] or in the event of smaller quantities to preparative-layer radial chromatography using Harrison research model 7924T chromatotrons with silica coated plates (Merck 60, PF$_{254}$, 7749).
3.4: Extractives from *Ocotea kenyensis*.

Crushed and dried leaves (130g) and milled bark (840g) were extracted with CH$_2$Cl$_2$ at room temperature for two days before the solvent was removed to give a green gum (3.81g) and a black gum (12.48g) respectively. A similar technique to that above was followed except that the initial solvent used in the plug was 100% chloroform.
Iso-ocobullenone.

\[ \text{Iso-ocobullenone,} \]

A fraction of the initial plug was subjected to further column chromatography in hexane-ether (7:5) to give iso-ocobullenone, $\Delta^8\text{-}$5-methoxy-3,4-methyleneedioxy-3',4'-methylenedioxy-1',2',3',6'-tetrahydro-6'-oxo-7',1',8',3'-neolignan \((I)\) (138 mg (bark), 37 mg (leaves)), mp138°C (lit. value 142-4°C [74]), $\alpha_D^{25} = +96.6^\circ$ (CHCl$_3$; c=0.025), lit. value $\alpha_D=122.6$ (CHCl$_3$, c=0.05), $^1$H NMR: 1.18 (3H, d, $J=6.90$ Hz, H-9), 2.08 (1H, dd, $J=14.20$, 8.89Hz, H-7'a), 2.09 (1H, dd, $J=10.80$, 1.30 Hz, H-2'a), 2.35 (1H, d, $J=10.81$ Hz, H-2'b), 2.50 (1H, qd, $i=13.71$ Hz, H-8), 2.68 (1H, d, $J=5.89$ Hz, H-7), 3.88 (3H, s, OMe), 5.12 (2H, m, H-9'), 5.43 (1H, s, H-5'), 5.47 (2H, d, $J=0.32$ Hz, 3', 4'-methylenedioxy), 5.66 – 5.85 (1H, s, H-8'), 5.92 (2H, m, 3, 4 methylenedioxy), 6.25 (2H, m, H-2, H-6), $^{13}$C NMR: 16.95 (q, C-9), 37.14 (t, C-7'), 43.17 (t, C-2'), 43.61 (d, C-8), 56.66 (q, OMe), 58.16 (s, C-1'), 59.47 (d, C-7), 87.45 (s, C-3'), 96.98 (d, C-5'), 99.31 (t, aliph –OCH$_2$O–), 101.36 (t, arom –OCH$_2$O–), 102.20 (d, C-6), 108.67 (d, C-2), 118.48 (t, C-9'), 122.23 (s, C-1), 134.46 (d, C-8'), 134.51 (s, C-4), 142.96 (s, C-3), 179.14 (s, C-4'), 198.70 (s, C-6'), high resolution EiMS 370.1420, required for C$_{21}$H$_{22}$O$_6$ 370.1415.
Plug fractions from the dichloromethane extract were combined and subjected to column chromatography using ether-hexane (5:7) as eluant. Two successive columns gave bullatone, $\Delta^8$-3,4,5-trimethoxy-3',6'-dihydro-3',4'-methylenedioxy-6'-oxo-8,3'-neolignan (3), as a yellow paste (32 mg (leaves), 69 mg (bark), $[\alpha]_D^{25} = 156^\circ$ (CHCl$_3$, c=0.09), lit. value $159.4^\circ$ [74], $^1$H NMR $\delta$(ppm): 0.88 (3H, $d$, $J=6.7$ Hz, Me-9), 1.78 (1H, $dd$, $J=12.7$ Hz, H-2b), 2.09 (1H, $ddq$, $J=12.6$, 6.8, 2.6 Hz, H-8), 2.25 (1H, $dd$, $J=12.6$, 11.8 Hz, H-7'b), 2.26 (1H, $m$, H-7'b), 2.40 (1H, $m$, H-1'), 2.56 (1H, $dd$, $J=5.2$ Hz, H-2'), 2.69 (1H, $m$, H-7'a), 2.87 (1H, $dd$, $J=12.6$, 2.4 Hz, H-7a), 3.80 (3H, $s$, OMe-4), 3.82 (3H, $s$, OMe-3, 5), 5.07 (2H, $m$, H-9'), 5.55 (1H, $s$, H-5''), 5.60 (2H, $dd$, $J=7.9$ Hz, -CH$_2$OCH$_2$-), 5.70 (1H, $m$, H-8'), 6.27 (2H, $s$, H-2, 6), $^{13}$C NMR: 15.10 (q, C-9), 36.48 ($t$, C-2'), 36.72 ($t$, C-7'), 39.01 ($t$, C-7), 42.50 ($d$, C-8), 42.64 ($d$, C-1'), 56.40 ($q$, OMe-3), 56.70 ($q$, OMe-5), 61.20 ($q$, OMe-4), 82.59 ($s$, C-3'), 99.34 ($d$, C-5'), 100.9 ($t$, -OCH$_2$O-), 106.19 ($d$, C-6), 106.24 (C-2), 117.52 ($t$, C-9'), 136.94 ($s$, C-4), 153.03 ($s$, C-5), 153.23 ($s$, C-5), 179.44 ($d$, C-4'), 198.80 ($s$, C-6'), high resolution EIMS 370.1406, required for C$_{21}$H$_{28}$O$_6$ $-$ 370.1416.
\[ \Delta^8\cdot 3,5\text{-dimethoxy-3',4'-methyleneoxy-1',2',3',4'-tetrahydro-6'-oxo-7,1',8,3'-neolignan}. \]

The fraction above also gave a small amount of semi-crystalline paste (3 mg) which was identified as \( \Delta^8\cdot 3,5\text{-methoxy-3',4'-methyleneoxy-1',2',3',4'-tetrahydro-6'-oxo-7,1',8,3'-neolignan} \), \( ^{1} \)H NMR \( \delta \) (ppm): 1.19 (3H, \( d, J=6.90 \) Hz, H-9), 2.07 (2H, \( dd, J=14.25, 8.90 \) Hz, H-7'), 2.08 (1H, \( dd, J=10.81, 1.28 \) Hz, H-2'a), 2.33 (1H, \( d, J=10.85 \) Hz, H-2'b), 2.48 (1H, \( dq, J=13.7 \) Hz, H-8), 2.68 (1H, \( d, J=5.90 \) Hz, H-7), 3.81 (3H, s, 5-OMe), 3.89 (3H, s, 3-OMe), 5.07 – 5.18 (2H, \( m, H-9' \)), 5.43 (1H, s, H-5'), 5.47 and 5.73 (2H, \( d, 3',4'\text{-methyleneoxy} \)), 5.66 – 5.85 (1H, \( m, H-8' \)), 6.30 (2H, s, H-2, 6), 6.44 (1H, s, H-4), \(^{13} \)C NMR \( \delta \) (ppm): 17.0 (q, C-9), 37.1 (t, C-7'), 43.5 (t, C-2'), 43.8 (d, C-8), 56.0 (q, OMe x 2), 58.1 (s, C-1'), 59.9 (d, C-7), 87.8 (s, C-3'), 96.8 (d, C-5'), 99.3 (t, OCH_2O), 105.8 (d, C-4), 105.9 (s, C-6, 2), 119.4 (C-9'), 133.1 (C-8'), 134.5 (s, C-1), 153.2 (s, C-3, 5), 179.4 (s, C-4'), high resolution EIMS 356.1618 (required for C_{21}H_{24}O_{5}, 356.1624).
The first fraction of the plug was subjected to column chromatography using chloroform-ethyl acetate (1:0.02) to give, (159mg) β-sitosterol, (24-Ethylcholest-5-ene-3β-ol) (21), mp139-141°C (lit. value 136-137°C [108]), [α]D = -34.0° (c, 0.050 in CHC1₃, 25°C), ¹³C NMR: δ(ppm) See table. ¹H NMR: δ(ppm) 0.68 (3H, s, H-18), 0.78 (3H, d, H-27), 0.80 (3H, d, H-26), 0.82 (3H, t, H-29), 0.91 (3H, d, H-21), 0.99 (3H, s, H-19), 3.50 (1H, m, H-3α), 5.32 (1H, m, H-6); ¹³C NMR: See table 3.
<table>
<thead>
<tr>
<th>Carbon atom</th>
<th>3</th>
<th>Literature values</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>37.2</td>
<td>37.2</td>
</tr>
<tr>
<td>2</td>
<td>31.6</td>
<td>31.6</td>
</tr>
<tr>
<td>3</td>
<td>71.8</td>
<td>71.8</td>
</tr>
<tr>
<td>4</td>
<td>42.3</td>
<td>42.5</td>
</tr>
<tr>
<td>5</td>
<td>140.7</td>
<td>140.7</td>
</tr>
<tr>
<td>6</td>
<td>121.7</td>
<td>121.7</td>
</tr>
<tr>
<td>7</td>
<td>31.9</td>
<td>31.8</td>
</tr>
<tr>
<td>8</td>
<td>31.9</td>
<td>31.9</td>
</tr>
<tr>
<td>9</td>
<td>50.1</td>
<td>50.1</td>
</tr>
<tr>
<td>10</td>
<td>36.5</td>
<td>36.5</td>
</tr>
<tr>
<td>11</td>
<td>21.1</td>
<td>21.1</td>
</tr>
<tr>
<td>12</td>
<td>39.7</td>
<td>39.7</td>
</tr>
<tr>
<td>13</td>
<td>42.3</td>
<td>42.3</td>
</tr>
<tr>
<td>14</td>
<td>56.7</td>
<td>56.7</td>
</tr>
<tr>
<td>15</td>
<td>24.3</td>
<td>24.3</td>
</tr>
<tr>
<td>16</td>
<td>28.3</td>
<td>28.3</td>
</tr>
<tr>
<td>17</td>
<td>56.0</td>
<td>56.0</td>
</tr>
<tr>
<td>18</td>
<td>11.9</td>
<td>11.9</td>
</tr>
<tr>
<td>19</td>
<td>19.4</td>
<td>19.4</td>
</tr>
<tr>
<td>20</td>
<td>36.1</td>
<td>36.1</td>
</tr>
<tr>
<td>21</td>
<td>18.8</td>
<td>18.8</td>
</tr>
<tr>
<td>22</td>
<td>33.9</td>
<td>33.9</td>
</tr>
<tr>
<td>23</td>
<td>26.0</td>
<td>26.0</td>
</tr>
<tr>
<td>24</td>
<td>45.8</td>
<td>45.8</td>
</tr>
<tr>
<td>25</td>
<td>29.1</td>
<td>29.1</td>
</tr>
<tr>
<td>26</td>
<td>19.8</td>
<td>19.8</td>
</tr>
<tr>
<td>27</td>
<td>19.0</td>
<td>19.1</td>
</tr>
<tr>
<td>28</td>
<td>23.0</td>
<td>23.0</td>
</tr>
<tr>
<td>29</td>
<td>12.0</td>
<td>12.0</td>
</tr>
</tbody>
</table>

Table 3
Triacontane.

CH$_3$(CH$_2$)$_{28}$CH$_3$

The first portion of the plug on the leaves of *Ocotea kenyensis* yielded triacontane (22) as a crystalline solid (68mg) mp 54 °C lit. value 65.9 °C [109], $^1$H NMR: 0.88 (t, H-1, 30), 1.26 (m, H-2 →29), $^{13}$C NMR: 14.14 (q, C-1, 30), 22.71 (t, C-2, 29) 29.38 (t, C-8, 23), 29.71 (m, C-4, 5, 6, 7, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 24, 25, 26, 27), 31.94 (t, C-3, 28).
The same portion as above yielded \((E,E,Z,Z,Z,Z,Z,Z,Z,Z)-\text{undecaprenol}\) (6), as a clear oil (39 mg). \(^1\)H NMR: \(\delta\) (ppm) 1.60 (s, 9H, H-45, 46, 47), 1.68 (s, 24H, H-44, 48, 49, 50, 51, 52, 53, 54), 1.74 (s, 3H, H-55), 2.03 (m, 41H, H-4, 5, 8, 9, 12, 13, 16, 17, 20, 21, 24, 25, 28, 29, 32, 33, 36, 37, 40, 41, OH), 4.10 (d, 2H, J=5.9Hz, H-1), 5.12 (br. s, 10H, H-6, 10, 14, 18, 22, 26, 30, 34, 38, 42), 5.45 (t, 1H, H-2), \(^{13}\)C NMR \(\delta\)(ppm): 16.00 (q, C-46, 47), 23.44 (q, C-48, 49, 50, 51, 52, 53, 54, 55), 25.71 (q, C-45), 23.44 (t, C-5, 9, 13, 17, 21, 25, 29), 32.20 (t, C-33, 37, 41), 39.73 (t, C-4, 8, 12, 16, 20, 24, 28, 32, 36, 40), 58.99 (t, C-1), 124.40 (d, C-42), 124.88 (d, C-38), 124.94 (d, C-34), 124.94 (d, C-2), 125.01 (d, C-6, 10, 14, 18, 22, 26, 30), 131.23 (d, C-43), 134.87 (s, C-39), 134.94 (s, C-35), 135.18 (s, C-7, 11, 15, 19, 23, 27, 31), 139.81 (s, C-3).
The same portion of the plug that yielded the previous two compounds gave a third compound that has been assigned the provisional structure below. 2-Undecaisoprenone (7) was isolated as a clear oil (24 mg) from the leaves. $^1$H NMR $\delta$(ppm): 1.59 (s, 30H, H-45, 46, 47, 48, 49, 50, 51, 52, 53, 54), 1.68 (s, 3H, H-44), 2.02 (m, 42H, H-8, 9, 12, 13, 16, 17, 20, 21, 24, 25, 28, 29, 32, 33, 36, 37, 40, 41; also 1 and 55), 3.11 (d of $t$, 2H, H-5), 5.11 (m, 10H, H-6, 10, 14, 18, 22, 26, 30, 34, 38, 42), 6.48 (t, 1H, H-3), $^{13}$C NMR $\delta$(ppm): 16.03 (q, C-45, 46, 47, 48, 49, 50, 51, 52, 53, 54) 16.15 (q, C-55), 17.69 (q, C-45), 25.71 (q, C-44), 26.69 (t, C-9, 13, 17, 21, 25, 29, 33, 37, 41), 27.47 (q, C-1), 29.71 (t, C-6), 39.75 (t, C-8, 12, 16, 20, 24, 28, 32, 36, 40), 118.09 (d, C-6), 123.80 (d, C-42), 124.24 (d, C-10, 14, 18, 22, 26, 30, 34, 38), 132.00 (s, C-43), 134.92 (s, C-11, 15, 19, 23, 27, 31, 35, 39), 139.70 (s, C-7), 147.94 (s, C-4).
3.5: Extractives from *Apodytes dimidiata*.

Leaves (1.11 kg) were crushed with a mortar and pestle and extracted with dichloromethane for 24h at room temperature. The plant material was filtered off and the extract was dried over MgSO₄. The solvent was removed under vacuum to give a pasty green extract (17.1g). This was applied to a plug of silica gel and eluted with ethyl acetate-hexane (2:1) and a solvent gradient up to 100% methanol. The same combination technique described above was used.

**Lupeol.**

The dichloromethane extract above was subjected to column chromatography using diethyl ether-hexane (2:1) to give 4, (87mg), lupeol, (20-(29)-lupen-3β-ol) (II) which showed complete agreement in its NMR data with those of the literature, mp178 °C (lit. value 215-216°C) [110], ¹H NMR: δ(ppm) 0.76 (3H, s, H-23), 0.79 (3H, s, H-28), 0.83 (3H, s, H-24), 0.94 (3H, s H-27), 0.97 (3H, s, H-25), 1.03 (3H, s, H-26), 1.68
(3H, s, H-29), 2.38 (1H, d of t, H-13), 3.19 (1H, m, H-3), 4.57 (1H, m, H-30a), 4.68 (1H, d of d of d, H-30b), $^{13}$C NMR: see table 4, [M$^+$] $\lambda$ 427 (C$_{30}$H$_{50}$O).
<table>
<thead>
<tr>
<th>Carbon atom</th>
<th>Lupeol</th>
<th>Lit. values</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>38.7</td>
<td>38.7</td>
</tr>
<tr>
<td>2</td>
<td>27.4</td>
<td>27.5</td>
</tr>
<tr>
<td>3</td>
<td>79.0</td>
<td>79.0</td>
</tr>
<tr>
<td>4</td>
<td>38.8</td>
<td>38.9</td>
</tr>
<tr>
<td>5</td>
<td>55.3</td>
<td>55.3</td>
</tr>
<tr>
<td>6</td>
<td>18.3</td>
<td>18.3</td>
</tr>
<tr>
<td>7</td>
<td>34.3</td>
<td>34.3</td>
</tr>
<tr>
<td>8</td>
<td>40.8</td>
<td>40.9</td>
</tr>
<tr>
<td>9</td>
<td>50.4</td>
<td>50.5</td>
</tr>
<tr>
<td>10</td>
<td>37.2</td>
<td>37.2</td>
</tr>
<tr>
<td>11</td>
<td>20.9</td>
<td>21.0</td>
</tr>
<tr>
<td>12</td>
<td>25.1</td>
<td>25.2</td>
</tr>
<tr>
<td>13</td>
<td>38.0</td>
<td>38.1</td>
</tr>
<tr>
<td>14</td>
<td>42.8</td>
<td>42.9</td>
</tr>
<tr>
<td>15</td>
<td>27.4</td>
<td>27.5</td>
</tr>
<tr>
<td>16</td>
<td>35.6</td>
<td>35.6</td>
</tr>
<tr>
<td>17</td>
<td>43.0</td>
<td>43.0</td>
</tr>
<tr>
<td>18</td>
<td>48.0</td>
<td>48.0</td>
</tr>
<tr>
<td>19</td>
<td>48.3</td>
<td>48.3</td>
</tr>
<tr>
<td>20</td>
<td>150.9</td>
<td>150.9</td>
</tr>
<tr>
<td>21</td>
<td>29.8</td>
<td>29.9</td>
</tr>
<tr>
<td>22</td>
<td>40.0</td>
<td>40.0</td>
</tr>
<tr>
<td>23</td>
<td>28.0</td>
<td>28.0</td>
</tr>
<tr>
<td>24</td>
<td>15.3</td>
<td>15.3</td>
</tr>
<tr>
<td>25</td>
<td>16.1</td>
<td>16.1</td>
</tr>
<tr>
<td>26</td>
<td>16.0</td>
<td>16.0</td>
</tr>
<tr>
<td>27</td>
<td>14.5</td>
<td>14.6</td>
</tr>
<tr>
<td>28</td>
<td>18.0</td>
<td>18.0</td>
</tr>
<tr>
<td>29</td>
<td>19.3</td>
<td>19.3</td>
</tr>
<tr>
<td>30</td>
<td>109.3</td>
<td>109.3</td>
</tr>
</tbody>
</table>

Table 4
A plug of the CH$_2$Cl$_2$ extract, run in ethyl acetate-hexane (2:1), gave a yellow gum (1.2g) from the portion collected at the solvent front. This was subjected to column chromatography in ether-petroleum ether (1:10) to give squalene, (2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosanehexaene) (10), (164 mg), as a clear oil. $^1$H NMR: $\delta$(ppm) 1.60 (18H, $m$, H-25,26,27,28,29,30), 1.67 (6H, $m$, H-1,24), 2.17 (20H, $m$, H-4,5,8,9,12,13,16,17,20,21), 5.10 (6H, $m$, H-3,7,11,14,18,22), $^{13}$C NMR: $\delta$(ppm) 16.00 ($q$, C-27, C-28), 16.05 ($q$, C-26, C-29), 17.69 ($q$, C-25, C-30), 25.71 ($q$, C-1, C-24), 26.65 ($t$, C-8, C-17), 26.76 ($t$, C-4, C-21), 28.28 ($t$, C-12, C-13), 39.74 ($t$, C-5, C-9, C-16, C-20), 124.27 ($d$, C-11, C-14), 124.30 ($d$, C-7, C-18), 124.40 ($d$, C-3, C-22), 131.24 ($s$, C-2, C-23), 134.88 ($s$, C-10, C-15), 135.09 ($s$, C-6, C-19) [M$^+$] 410 (C$_{30}$H$_{50}$).
Betulinic acid (12), precipitated *en masse* from the latter, more polar portion of the plug and was recrystallised from methanol to give a white powder (308 mg) mp 277°C, lit. values 275-278°C [111], found C 78.65; H 10.61, calculated C 78.90; H 10.59. $^1$H NMR δ(ppm) (pyridine-d$_6$) 0.68 (s, 3H, H-23), 0.85 (s, 3H, H-24), 0.90 (s, 3H, H-25), 1.07 (s, 3H, H-26), 0.93 (s, 3H, H-27), 1.65 (s, 3H, H-30), 3.35 (m, 2H, H-3, 19), 4.63 (d, 1H, J=2.11 Hz, H-29a), 4.79 (d, 1H, J=2.14Hz, H-29b).

$^{13}$C NMR δ(ppm), (pyridine-d$_6$): See table 5.
<table>
<thead>
<tr>
<th>Carbon atom</th>
<th>Literature value</th>
<th>Shift value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>38.7</td>
<td>36.7</td>
</tr>
<tr>
<td>2</td>
<td>27.4</td>
<td>27.3</td>
</tr>
<tr>
<td>3</td>
<td>78.9</td>
<td>77.3</td>
</tr>
<tr>
<td>4</td>
<td>38.8</td>
<td>40.1</td>
</tr>
<tr>
<td>5</td>
<td>55.3</td>
<td>55.1</td>
</tr>
<tr>
<td>6</td>
<td>18.3</td>
<td>17.8</td>
</tr>
<tr>
<td>7</td>
<td>34.3</td>
<td>32.0</td>
</tr>
<tr>
<td>8</td>
<td>40.7</td>
<td>40.2</td>
</tr>
<tr>
<td>9</td>
<td>50.5</td>
<td>50.1</td>
</tr>
<tr>
<td>10</td>
<td>37.2</td>
<td>38.6</td>
</tr>
<tr>
<td>11</td>
<td>20.8</td>
<td>20.3</td>
</tr>
<tr>
<td>12</td>
<td>25.5</td>
<td>25.1</td>
</tr>
<tr>
<td>13</td>
<td>38.4</td>
<td>37.7</td>
</tr>
<tr>
<td>14</td>
<td>42.4</td>
<td>41.9</td>
</tr>
<tr>
<td>15</td>
<td>30.5</td>
<td>29.4</td>
</tr>
<tr>
<td>16</td>
<td>32.1</td>
<td>33.9</td>
</tr>
<tr>
<td>17</td>
<td>56.3</td>
<td>55.7</td>
</tr>
<tr>
<td>18</td>
<td>46.8</td>
<td>46.9</td>
</tr>
<tr>
<td>19</td>
<td>49.2</td>
<td>48.9</td>
</tr>
<tr>
<td>20</td>
<td>150.3</td>
<td>150.7</td>
</tr>
<tr>
<td>21</td>
<td>29.7</td>
<td>30.3</td>
</tr>
<tr>
<td>22</td>
<td>37.0</td>
<td>38.4</td>
</tr>
<tr>
<td>23</td>
<td>27.9</td>
<td>27.8</td>
</tr>
<tr>
<td>24</td>
<td>15.3</td>
<td>15.4</td>
</tr>
<tr>
<td>25</td>
<td>16.0</td>
<td>15.4</td>
</tr>
<tr>
<td>26</td>
<td>16.1</td>
<td>14.5</td>
</tr>
<tr>
<td>27</td>
<td>14.7</td>
<td>14.0</td>
</tr>
<tr>
<td>28</td>
<td>180.5</td>
<td>178.3</td>
</tr>
<tr>
<td>29</td>
<td>109.6</td>
<td>109.3</td>
</tr>
<tr>
<td>30</td>
<td>19.4</td>
<td>18.6</td>
</tr>
</tbody>
</table>

**Table 5**

Sucrose was isolated from the methanol extract and identified as the octa acetate.
Catechin (I3) was isolated from the subsequent methanol extraction of *Apodytes dimidiata* after the methanol had been removed to give (19.50 g) of dark gum. The application of column chromatography to a portion (3g) of the above, using chloroform: methanol (3:1), gave 9 (72 mg), as a semi-crystalline paste, $^1$H NMR $\delta$(ppm): 2.52 ($dd$, $J=16.1$, 8.2 Hz, 1H, H-4a), 2.89 ($dd$, 1H, $J=16.2$, 5.5 Hz, H-4b), 4.01 ($t$ of $d$, 1H, $J=7.8$, 5.5 Hz, H-3), 4.60 ($d$, 1H, $J=7.5$ Hz, H-2), 5.89 ($d$, 1H, $J=2.3$ Hz, H-5), 5.96 ($d$, 1H, $J=2.3$Hz, H-7), 6.74 ($d$, 1H, $J=1.8$ and 7.50Hz, H-2'), 6.78 ($d$, 1H, $J=7.49$ Hz, H-6'), 6.88 ($d$, 1H, $J=1.8$ Hz, H-3'), $^{13}$C NMR $\delta$(ppm) (CD$_3$OD): 28.5 ($t$, C-4), 68.8 ($d$, C-3), 82.8 ($d$, C-2), 95.5 ($d$, C-7), 96.3 ($d$, C-5), 115.2 ($d$, C-3'), 116.0 ($d$, C-2'), 120.0 ($d$, C-6'), 132.1 (s, C-10), 146.2 (s, C-9), 154.28 (s, C-8), 156.9 (s, C-4'), 157.5 (s, C-5'), 157.7 (s, C-6).
The methanol extract also gave benzoic acid (20), (590 mg) from another portion of the plug which was placed on a column using ether-chloroform (2:1), mp 120°C (lit. value 122°C) [93], $^1$H NMR: $\delta$(ppm) 7.48 (2H, m, H-3, H-5), 7.62 (1H, t of t, H-4), 8.14 (2H, m, H-2, H-6), 11.10 (1H, broad s, OH), $^{13}$C NMR: $\delta$(ppm) 128.49 ($d$, C-3, C-5), 129.30 ($d$, C-1), 130.21 ($d$, C-2, C-6), 133.83 ($d$, C-4), 172.50 ($s$, C-7).
3.6: Extractives from *Ehretia rigida*.

Milled bark (1.34 kg) was extracted according to the general procedure to yield: 8.87g hexane extract, 7.34g chloroform extract and 9.40g methanol extract. The ethyl acetate extract was subjected to a partitioning against water once it had been concentrated down (to ca. 300ml) under vacuum to give 3.95g dark red gum.

**Allantoin.**

\[
\begin{align*}
\text{H} & \quad \text{O} & \quad \text{O} & \quad \text{N} & \quad \text{N} \\
\text{H} & \quad \text{N} & \quad \text{N} & \quad \text{H} \\
\text{H} & \quad \text{H} & \quad \text{H} & \quad \text{H}
\end{align*}
\]

\[(17)\]

The methanol fraction was concentrated to ca.100ml with the formation of a precipitate that was filtered off to give a white powder (0.35g). This powder was recrystallised from water and 17 was obtained (ca.200mg). Allantoin (N-(2,5-dioxo-4-imidazolidinyl)urea) mp 205°C (literature value 226-9°C [112]), \(^1\)H NMR: \(\delta\) (ppm) 5.23 (1H, q, H-4), 5.80 (2H, s, H-8), 6.89 (1H, d, H-3), 8.06 (1H, s, H-1), 10.54 (1H, s, H-6), \(^{13}\)C NMR: \(\delta\) (ppm) 62.46 (d, C-4), 156.83 (s, C-7), 157.42 (s, C-2), 173.67 (s, C-5), MS direct probe \(m/z\) (M\(^+\))157.61.
TSP2 was found in very large quantities (more than 1% of the dry weight of the bark) in the hexane (ca. 10 g) and chloroform (ca. 3 g) extracts but was incompletely separated into its three components. The compounds co-precipitated from the hexane extract as the solvent was being removed and were filtered at this stage. Recrystallisation from hexane to give white needles mp 185°C. Co-crystallization had occurred. The major component of the mixture is β-amyrin (19) mp lit. value 197-197.5°C [113]; $^1$H NMR δ(ppm): 0.90 – 2.30 indiscernible due to the mixture of compounds present, 3.25 (1H, $m$, H-3), 5.12 (1H, t, H-12).

$^{13}$C NMR: See table 6.
<table>
<thead>
<tr>
<th>Carbon Atom</th>
<th>TSP2</th>
<th>Literature value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>38.7</td>
<td>38.7</td>
</tr>
<tr>
<td>2</td>
<td>27.2</td>
<td>27.3</td>
</tr>
<tr>
<td>3</td>
<td>79.0</td>
<td>79.0</td>
</tr>
<tr>
<td>4</td>
<td>38.8</td>
<td>38.8</td>
</tr>
<tr>
<td>5</td>
<td>55.1</td>
<td>55.3</td>
</tr>
<tr>
<td>6</td>
<td>18.4</td>
<td>18.5</td>
</tr>
<tr>
<td>7</td>
<td>32.9</td>
<td>32.8</td>
</tr>
<tr>
<td>8</td>
<td>38.8</td>
<td>38.8</td>
</tr>
<tr>
<td>9</td>
<td>47.7</td>
<td>47.7</td>
</tr>
<tr>
<td>10</td>
<td>37.7</td>
<td>37.6</td>
</tr>
<tr>
<td>11</td>
<td>23.5</td>
<td>23.6</td>
</tr>
<tr>
<td>12</td>
<td>121.7</td>
<td>121.8</td>
</tr>
<tr>
<td>13</td>
<td>145.1</td>
<td>145.1</td>
</tr>
<tr>
<td>14</td>
<td>41.7</td>
<td>41.8</td>
</tr>
<tr>
<td>15</td>
<td>26.1</td>
<td>26.2</td>
</tr>
<tr>
<td>16</td>
<td>27.1</td>
<td>27.0</td>
</tr>
<tr>
<td>17</td>
<td>32.5</td>
<td>32.5</td>
</tr>
<tr>
<td>18</td>
<td>47.6</td>
<td>47.4</td>
</tr>
<tr>
<td>19</td>
<td>46.8</td>
<td>46.9</td>
</tr>
<tr>
<td>20</td>
<td>31.1</td>
<td>31.1</td>
</tr>
<tr>
<td>21</td>
<td>34.7</td>
<td>34.8</td>
</tr>
<tr>
<td>22</td>
<td>37.1</td>
<td>37.2</td>
</tr>
<tr>
<td>23</td>
<td>28.1</td>
<td>28.2</td>
</tr>
<tr>
<td>24</td>
<td>15.5</td>
<td>15.5</td>
</tr>
<tr>
<td>25</td>
<td>15.6</td>
<td>15.6</td>
</tr>
<tr>
<td>26</td>
<td>16.8</td>
<td>16.9</td>
</tr>
<tr>
<td>27</td>
<td>25.9</td>
<td>26.0</td>
</tr>
<tr>
<td>28</td>
<td>28.4</td>
<td>28.4</td>
</tr>
<tr>
<td>29</td>
<td>33.3</td>
<td>33.3</td>
</tr>
<tr>
<td>30</td>
<td>23.7</td>
<td>23.7</td>
</tr>
</tbody>
</table>

**Table 6**
The next most abundant component of the mixture is α-amyrin (I8) mp lit. value 186°C [114]; \(^{1}H\) NMR δ(ppm): 0.90 – 2.30 indiscernible due to the mixture of compounds present, 3.25 (1H, m, H-3), 5.19 (1H, t, H-12).

\(^{13}C\) NMR: See table 7.
<table>
<thead>
<tr>
<th>Carbon atom</th>
<th>TSP2</th>
<th>Literature value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>38.7</td>
<td>38.7</td>
</tr>
<tr>
<td>2</td>
<td>27.2</td>
<td>27.2</td>
</tr>
<tr>
<td>3</td>
<td>78.9</td>
<td>78.3</td>
</tr>
<tr>
<td>4</td>
<td>38.6</td>
<td>38.7</td>
</tr>
<tr>
<td>5</td>
<td>55.1</td>
<td>55.2</td>
</tr>
<tr>
<td>6</td>
<td>18.3</td>
<td>18.3</td>
</tr>
<tr>
<td>7</td>
<td>32.6</td>
<td>32.9</td>
</tr>
<tr>
<td>8</td>
<td>40.0</td>
<td>40.0</td>
</tr>
<tr>
<td>9</td>
<td>47.6</td>
<td>47.7</td>
</tr>
<tr>
<td>10</td>
<td>36.9</td>
<td>36.9</td>
</tr>
<tr>
<td>11</td>
<td>23.2</td>
<td>23.3</td>
</tr>
<tr>
<td>12</td>
<td>124.3</td>
<td>124.3</td>
</tr>
<tr>
<td>13</td>
<td>139.5</td>
<td>139.3</td>
</tr>
<tr>
<td>14</td>
<td>42.0</td>
<td>42.0</td>
</tr>
<tr>
<td>15</td>
<td>28.7</td>
<td>28.7</td>
</tr>
<tr>
<td>16</td>
<td>26.6</td>
<td>26.6</td>
</tr>
<tr>
<td>17</td>
<td>33.7</td>
<td>33.7</td>
</tr>
<tr>
<td>18</td>
<td>59.0</td>
<td>58.9</td>
</tr>
<tr>
<td>19</td>
<td>39.6</td>
<td>39.6</td>
</tr>
<tr>
<td>20</td>
<td>39.6</td>
<td>39.6</td>
</tr>
<tr>
<td>21</td>
<td>31.2</td>
<td>31.2</td>
</tr>
<tr>
<td>22</td>
<td>41.5</td>
<td>41.5</td>
</tr>
<tr>
<td>23</td>
<td>28.1</td>
<td>28.1</td>
</tr>
<tr>
<td>24</td>
<td>15.6</td>
<td>15.6</td>
</tr>
<tr>
<td>25</td>
<td>15.7</td>
<td>15.6</td>
</tr>
<tr>
<td>26</td>
<td>16.8</td>
<td>16.8</td>
</tr>
<tr>
<td>27</td>
<td>23.3</td>
<td>23.3</td>
</tr>
<tr>
<td>28</td>
<td>28.1</td>
<td>28.1</td>
</tr>
<tr>
<td>29</td>
<td>17.5</td>
<td>17.4</td>
</tr>
<tr>
<td>30</td>
<td>21.4</td>
<td>21.3</td>
</tr>
</tbody>
</table>

**Table 7**

High resolution EIMS: observed m/z 426.3861, C$_{30}$H$_{50}$O requires 426.3864, observed 218.2034, C$_{16}$H$_{26}$ requires 218.2034 (this is a product of a reverse Diels-Alder reaction and is the most abundant ion).
1-Triacontanol.

CH₃(CH₂)₂₈CH₂OH

The chloroform extract above (7.34g) was subjected to column chromatography using hexane-ether (2:1) to give 22, 1-triacontanol, (39mg) as white needles from MeOH/Ether, mp 85-7°C (lit. value 86.5°C) [115], ¹H NMR: δ(ppm) 0.88 (3H, t, H-30), 1.25 (52H, m, H-4→H-28), 1.58 (4H, m, H-3, H-29), 3.64 (2H, t, H-1), ¹³C NMR: δ(ppm) 14.14 (q, C-30), 22.71 (t, C-29), 25.75 (t, C-3), 29.38 (t, C-5), 29.45 (t, C-26) 29.62 (t, C-4, C-27) 29.67 (t, C-6, C-25) 29.71 (t, C-7→C-24) 31.94 (t, C-28) 32.81 (t, C-2), 63.11 (t, C-1).
Synthesis of 4-ethyl catechol.

Synthesis of 3,4-methylenedioxoy styrene (saffrole) by the Wittig reaction.

\[
\begin{align*}
\text{H}^a & \quad \text{H}^b \\
n & \quad 4 \\
3 & \quad 2 \\
\text{O} & \quad \text{O}
\end{align*}
\]

(24)

Methyl iodide (35.5 g) in ether was added to triphenylphosphine (65 g) in ether and stirred at 0°C overnight. The resulting precipitate was filtered and oven dried to give methyltriphenylphosphonium iodide (98.7 g). To a portion of this (85.0 g) in dry DMSO (210 ml) was added NaH under nitrogen and the mixture was stirred at room temperature for one hour. Piperonal (30.5 g) in THF was added under nitrogen and the mixture was stirred for another hour at room temperature. The reaction was quenched with wet MeOH and poured into H₂O. The water was extracted three times with CHCl₃ and the combined extracts concentrated down under vacuum. Column chromatography in the form of a plug run in hexane afforded 3,4-methylenedioxy styrene (24), (23.77 g, 79%) as a clear oil. \(^1\)H NMR: 6.95 (1H, m, H-3), 6.82 (1H, dd, H-5, J= 8.00Hz), 6.74 (1H, d, H-6, J= 7.99Hz), 6.61 (1H, d of d, H-8, J=17.53Hz, J'=10.84Hz), 5.93 (2H, s, H-7), 5.56 (1H, d, H-9c, J=17.56Hz), 5.11 (1H, d, H9b, J= 10.82Hz), \(^1\)C NMR: 147.97 (s, C-1), 147.33 (s, C-2), 136.34 (d, C-8), 132.09 (s, C-4), 121.00 (d, C-5), 111.95 (t, C-9), 108.17 (d, C-6), 105.36 (d, C-3), 101.03 (t, C-7), [M\(^+\)] 148.65.
Synthesis of 1,2-methylenedioxy-4-ethyl benzene.

To a 50 ml round bottomed flask containing 3,4-methylenedioxy styrene (2.42 g) in MeOH (10 ml) was added Pd on activated carbon (0.08 g, 10 mol %) under a hydrogen atmosphere and the mixture stirred overnight. The catalyst was filtered off using a Pasteur pipette with a plug of silica gel and the silica was washed with methanol / chloroform. The solvent was removed to give 1,2-methylenedioxy-4-ethyl benzene (25), (2.28 g, 93%) as a clear oil. $^1$H NMR: 6.65 (3H, m, H-3, H-5, H-6), 5.89 (2H, s, H-7), 2.53 (2H, q, H-8), 1.19 (3H, t, H-9), $^{13}$C NMR: 147.52 (s, C-1), 145.42 (s, C-2), 138.17 (s, C-4), 120.39 (d, C-5), 108.41 (d, C-3), 108.01 (d, C-6), 100.69 (t, C-7), 28.67 (t, C-8), 15.99 (q, C-9), m/z [M$^+$] 149.75.
Synthesis of 4-ethyl catechol.

To a flame dried two-necked round bottomed flask under an atmosphere of nitrogen was added dichloroethane (30 ml) and BCl$_3$.S(CH$_3$)$_2$ (1.43 g, 1.2 eq.). To this solution was added 1,2-methylenedioxy-4-ethyl benzene (1 g) and the reaction stirred at reflux and monitored by tlc. When the starting material was no longer visible on tlc ($\pm$4 hrs) the reaction was hydrolyzed by adding water ($\pm$30 ml) and stirring for 30 min. at room temperature while diluting with ether. The organic phase was separated and washed with 1M NaHCO$_3$ and the phenol was subsequently taken up with 1N NaOH (3x20 ml). The combined NaOH washings were acidified and the product was extracted into ether, dried (MgSO$_4$) and the solvent removed under vacuum to give 4-ethyl catechol (14), (0.59 g, 64%). $^1$H NMR: 6.75 (1H, m, H-3), 6.65 (2H, m, H-5, H-6), 5.90 (2H, broad s, 2xOH), 2.48 (2H, q, H-7), 1.13 (3H, t, H-8), $^{13}$C NMR: 143.22 (s, C-1), 141.04 (s, C-2), 137.82 (s, C-4), 120.39 (d, C-5), 115.60 (d, C-3), 115.27 (d, C-6), 28.10 (t, C-7), 15.68 (q, C-8), m/z [M$^+$] 137.95.
Bibliography


20. Personal Communication, Drewes, S. E.

21. Unpublished Personal Communication, Drewes, S. E.


73. Personal Communication, Drewes, S. E. with I.N.R.


96. Personal Communication, Drewes, S. E., 6th NAPRECA Conference, Kampala, Uganda.


101. Personal Communication, Drewes, S. E.


(7)
TSP2
Crystallographic data for Allantoin.

Formula

\[ \text{C}_4\text{H}_6\text{N}_4\text{O}_3 \]

Cell parameters

- a (angstroms) \(8.0126 (22)\)
- b (angstroms) \(5.1453 (14)\)
- c (angstroms) \(14.7715 (25)\)

\[ \alpha (\degree) \quad 90 \]
\[ \beta \quad 93.01 (2) \]
\[ \gamma \quad 90 \]

Volume (angstroms\(^3\)) \(608.14 (25)\)

Cell type

Monoclinic

Z

4

R factor

0.0368