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CHEMICAL STUDIES ON RESINS OF *BOSWELLIA PIROTTAE* AND *B. PAPYRIFERA*

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Chemical Studies on Resins of *Boswellia pirottae* and *B. papyrifera*

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APPROVAL SHEET

Addis Ababa University
College of Natural Sciences
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This is to certify that the thesis prepared by Sisay Awoke, entitled: Chemical Studies on Resins of *Boswellia pirottae* and *B. papyrifera*, submitted in partial fulfillment of the requirement of the degree of Doctor of Philosophy in Chemistry (Organic stream) complies with the regulation of the University and meets the accepted standards with respect to originality and quality.

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DECLARATION OF THE CANDIDATE

I, hereby, declare that this thesis submitted for the degree of Doctor of Philosophy (PhD) in Chemistry (Organic stream) at Addis Ababa University, College of Natural and Computational sciences, Addis Ababa, Ethiopia, is my own original work and has not been submitted previously to any institution or higher education. All sources of materials used in this work have been accordingly acknowledged.

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Contents

Table of figures v

Lists of tables vi

Acknowledgments viii

Abstract ix

Introduction 1

1. Highlights of an Asian and African *Boswellia* Species 1
  - 1.1. Botanical aspects 1
  - 1.2. Cultural, traditional and pharmacological significance 5
  - 1.3. Economic significance 6
  - 1.4. Chemical constituents of major species 7
  - 1.5. Biological activities of compounds isolated from frankincense 16
    - 1.5.1. Anti-inflammatory activities 16
    - 1.5.2. Anti-cancer and anti-tumor 17
    - 1.5.3. Apoptosis-regulating activities 17
  - 1.6. Aims of this dissertation 19
2. Results and discussion 20
  - 2.1. Specimen collection and identification 20
  - 2.2. Isolation and characterization of compounds from *Boswellia* resins 21
  - 2.3. Comparative studies of *Boswellia* species using qualitative TLC analysis 76
  - 2.4. Analysis of major components of essential oils of *Boswellia* spp. by GC-MS 85
  - 2.5. Quantification of AKBA and KBA in resin of *Boswellia* species by HPTLC 88
3. Conclusions 92
4. Experimental 94
  - 4.1. Resin materials, solvents and chemicals 94
  - 4.2. Extraction procedure 95
  - 4.3. Thin-layer and column chromatographic technique 97
  - 4.4. Fractionation and isolation of compounds from *Boswellia* resins 99

References 111

Appendixes 131

Table of figures

Figure 1: Distribution of *Boswellia* species in Ethiopia 4
Figure 2: Structures of monoterpenes in the resin of *Boswellia* species 9
Figure 3: Structures of diterpenes in the resin of *Boswellia* species 10
Figure 4: Structures of sesquiterpenes in the resin of *Boswellia* species 11
Figure 5a: Ursane- and oleanane-type triterpenes isolated from the *Boswellia* species 13
Figure 6: TLC of the EtOH extract of *B. papyrifera* Kebtele resin: a: neutral fraction (ss_1); b: acid fraction (ss_2) 22
Figure 7: TLC chromatogram of EtOH extract of *B. papyrifera* resin: (ss_1, ss_2) 41
Figure 8: TLC (Left: ss_1; Right: ss_2) chromatogram of EtOH extract of B. sacra 46
Figure 9: TLC (ss_1) chromatogram of the EtOH extract of *B. frereana* 48
Figure 10: TLC chromatogram of the EtOH extract of the resin of B. pirottae and isolated compounds (ss_1) 55
Figure 11: TLC chromatogram of EtOH extract of *B. neglecta* (ss_1) 64
Figure 12: TLC chromatogram of crude extracts and isolated compounds (ss_1) 66
Figure 13: TLC chromatograms of resines:neutral fraction (A), acid fraction (B), after irradiation with UV lamp at 256 nm (C) 78
Figure 14: TLC chromatograms of acid fractions of *B. papyrifera* (K) and *B. papyrifera* 79
Figure 15: TLC chromatograms of extracts of the resins of *B. sacra* (So = SolamLia, Ye = Yemen and Om = Oman) 80
Figure 16: Set-up of pyrolysis 82
Figure 17: TLC chromatogram of pyrolysates of four *Boswellia* species: 83
Figure 18: ^{13}C NMR data of pyrolysates of *Boswellia* species, cneorben-10-ol (119), verticill-3,7(20),11-triene (104), incensole (5), incensole (111) and serratol (108) 84
Figure 19: GC-MS Chromatograms of the oils of *Boswellia* resins 87
Figure 20: TLC chromatogram of spiked Bpap samples (a: AKBA and b: KBA) 89
Figure 21: TLC chromatogram and a calibration curve of AKBA (a, c) and KBA (b, d) 90
Figure 22: Three-dimensional spectral and spectra comparison of AKBA (a, b) KBA(c, d) 91
Figure 23: Photos of resins (By Professor Ermias Dagne) 95

Lists of tables

Table 1: *Boswellia* species and their geographical distribution 3

Table 2: Monoterpenes reported from *Boswellia* species 7

Table 3: Sesqui- and diterpenes reported from *Boswellia* species 10

Table 4: Ursane- and oleanane-type triterpinoids 12

Table 5: Lupane-, tirucallane-, dammarane-, euphane-, roburic- and canaric-type triterpenoids 14

Table 6: Percent yields of neutral and acid fractions 21

Table 7: ^{1}H and ^{13}C NMR spectral data of 104 and 119, and comparison with the data reported for verticilla-4(20),7,11-triene and cneorben-10-ol in the literature 24

Table 8: ^{1}H and ^{13}C NMR spectral data of 166 and 169, and comparison with the data reported for 3-acetyl tirucallic acid and 3-oxotirucallic acid in the literature 29

Table 9: ^{13}C NMR spectral data of 121, 122, 146 and 147, and comparison with the data reported for β-ABA, α-ABA, α-BA and β-BA in the literature 35

Table 10: ^{1}H and ^{13}C NMR spectral data of 123,124 and 132, and comparison with the data reported for AKBA, KBA and 11-hydroxy-ABA in the literature 37

Table 11: ^{1}H and ^{13}C NMR assignments for 108, 109 and 111, and comparison with the data reported for serratol, incensole and incensole acetate in the literature 43

Table 12: ^{1}H and ^{13}C NMR spectral data for 118 and comparison with the data reported for α-phellandrene dimer in the literature, 400 MHz, $CDCl_3$, δ in ppm, *mult*, *J* 49

Table 13: ^{1}H and ^{13}C NMR assignments for compounds 158, 159, 163, and 191, and comparison with the data reported for epilupeol, lupeol, epilupeol acetate and α-amyryl acetate in the literature (400 MHz, $CDCl_3$) 52

Table 14: NMR data of fatty acid esters triterpenes isolated from *B. pirottae (*400 MHz, $CDCl_3$) 57

Table 15: ^{13}C NMR spectral data of 148, 129, 157 and 201, and comparison with the data reported for α-amyrone, β-amyrone, lupenone and tirucall-8,24-dienone in the literature (400 MHz, $CDCl_3$) 59

Table 16: ^{13}C NMR spectral data of 130, 131, 149, 150, 170 and 201 and comparison of epi-*α*-amyrin, *α*-amyrin, *epi*-β-amyrin, β-amyrin, 3β-OH-tirucall-8, 24-diene and 3α-OH-tirucall-8, 24-diene (400 MHz, $CDCl_3$) 62

Table 17: ^{13}C NMR spectral data for 202 and comparison with the data reported for 3-β-OH-tirucall-7,24-diene (400 MHz, $CDCl_3$) 65
Table 18: ^{13}C NMR spectral data of compound 203-207 and 177, and comparison with the data for (20S)-20-hydroxydammar-24-en-diol acetate, 20S,24S- epoxydammarane -25-ol-yl acetate, (20S)-20-hydroxydammar-24-en-3-one, 20S,24S-epoxydammaran-25-ol-3-one, dammaranelactone-3α-acetate and (3β,20S)-dammar-24-endiol (400 MHz, $CDCl_3$) 70
Table 19: spectral comparison of 20- hydroxydammarenone I and II with 205 72
Table 20: Spectral comparison of ocotillone and cabraleone with 206 73
Table 21: TLC *Rf* values, solvent systems, colors of compounds identified from extracts of *Boswellia* resins 81
Table 22: Percentage of essential oils from *Boswellia* species 85
Table 23: Main components of essential oils from resins of *Boswellia* species 86
Table 24. Repeatability of the method (in µg) 88
Table 25. Results of recovery study 89
Table 26: Summary of quantitative TLC results 90
Table 27: Lists of Resins Investigated 94
Table 28: Amount of resins extracted (%) 95

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The information about a unique type of natural resin from a locality called Kebetele in North Ethiopia (Gojam Province) was communicated to our research group several years ago by the Natural Gum Processing and Marketing Enterprise (NGPME) of Ethiopia. The resin was then analyzed for the first time as part of an MSc thesis by Asmare in 2007 and further investigated in this dissertation. We wish to acknowledge here the NGPME for resin material and also for facilitating field work in the above site. Department of Chemistry Addis Ababa University and Wollo University are thanked for sponsorship.

Abstract

Chemical Studies on Resins of *Boswellia pirottae* and *B. papyrifera*

By Sisay Awoke

Advisor: Professor Ermias Dagne

The resins of *Boswellia* species enjoy a wide array of traditional uses such as for treatment of skin diseases, as incense and fumigation for religious and cultural activities, and as an anti-inflammatory in medicine.

This dissertation focuses on the chemical investigation of resins obtained from two *Boswellia* species, *namely, B. papyrifera* and *B. pirottae*. The B. papyrifera resin obtained from Kebtele showed chemically distinct from the resin obtained from known B. papyrifera resin. From this resin, two diterpenes (**104**, **119**), one fatty acid (**186**), two tirucallic acids (**169** and **166**), and seven boswellic acids (**121**,**122**, **123**, **124**, **132**, **146**, **147**) were isolated. However, from known *B. papyrifera*, two diterpenes (**111**, **109**) and one fatty acid (**190**) together with the same boswellic acids found in Kebtele resin were isolated.

The hitherto uninvestigated resin *B. pirottae*, an endemic species to Ethiopia, yielded eleven known triterpenes with different skeletons, three ursane (**129**, **130**, **131**), three oleanane (**157**, **149**, **150**), two lupane (**148**, **159**) and three tirucallane (**170**, **200**, **201**) derivatives.

However, in the remaining resins, *B. sacra*, *B. frereana*, *B. neglecta* and *B. rivae*, compounds that distingush one resin from the other have been isolated.

In this work, qualitative and quantitative analytical methods were developed using TLC, GC-MS, and HPTLC. The qualitative methods enabled the unequivocal identification of eight *Boswellia* species. The amount of the most potent anti-inflammatory and anti-cancer compounds found in the resin of *B. papyrifera* was compared with the well-known species, *B. serrata* and *B. sacra*. Accordingly, the amount of 11-keto-β-boswellic acid (**123**) and 3α-acetyl-11-keto-β-boswellic acid (**124**) in the EtOH extract of *B. papyrifera* were determined as 14.0% and 8%, respectively.

Frankincense resins are usually used as fumigants in cultural and religious ceremonies. We therefore analyzed the smokes of resins of *B. papyrifera*, *B. sacra* and *B. serrata* obtained by gentle burning and then capturing smoke constituents by extraction using solvents. Most of the compounds present in the resin were found in the smoke.

Lists of abbreviations

α-ABA	3-Acetyl- α-Boswellic acid
α-BA	α-Boswellic acid
β-ABA	3-Acetyl- β-boswellic acid
β-BA	β-Boswellic cid
ABAs	3-Acetylated-β- boswellic acid
AKBA	3-Acetyl-11-keto- β-boswellic acid
BpRn	*Boswellia papyrifera* resin neutral fraction
BpRa	*Boswellia papyrifera* resin acid fraction
BpKRn	*Boswellia papyrifera* Kebtele resin neutral fraction
BpKRa	*Boswellia papyrifera* Kebtele resin acid fraction
BsaRn	*Boswellia sacra* resin neutral fraction
BsaRa	*Boswellia sacra* resin acid fraction
BsRn	*Boswellia serrata* resin neutral fraction
BsRa	*Boswellia serrata* resin acid fraction
BfRn	*Boswellia* frereana resin neutral fraction
BfRa	*Boswellia* frereana resin acid fraction
BpiRn	*Boswellia pirottae* resin neutral fraction
BpiRa	*Boswellia pirottae* resin acid fraction
BnRn	*Boswellia neglecta* resin neutral fraction
BnRa	*Boswellia neglecta* resin acid fraction
BrRn	*Boswellia rivae* resin neutral fraction
BrRa	*Boswellia rivae* resin acid fraction
KBA	11-Keto- β-boswellic acid
Kebtele Resin	The resin of *B. papyrifera* collected from Kebtele (Awi zone, Jawe)
Markers	Compounds which exist as a specific or major component in the resin
Resin	*Boswellia* resin (Frankincense)
ss$_1$	Solvent system 1(Hexane: EtOAc / 4:1)
ss$_2$	Solvent system 2 (hexane: chloroform: EtOAc / 1:2:1)

Introduction

Frankincense is often associated with orthodox and Catholic churches because there it is burned on coal during spiritual ceremonies and emits a pleasant odor. It is one of the very important materials used as a component of offering made by fire to the Lord. As mentioned *"… and when any will offer a meat offering unto the Lord, his offering shall be of fine flour; and he shall pour oil upon it, and put frankincense thereon [1] [Leviticus 2:1-3,* Exodus 30:34-35*].* It is described more than 22 times in Holy Bible [1].

In addition to religious, cultural and traditional purposes, frankincense has also pharmacological applications. The anti-inflammatory action of alcohol extract of an Indian frankincense was discovered in the early 19th century. Since then, the pharmacological interest in extracts and single isolated compounds has been growing enormously [2-5].

In the following sections, review of the literature on botanical aspects, chemistry, pharmacological applications, cultural and economic significance of resins of *Boswellia* species, and brief review on biological activities of isolated compounds are described.

1. Highlights of an Asian and African *Boswellia* Species

1.1. Botanical aspects

Boswellia species are natural resin bearing plants. The natural oleogum resin that exudes from incisions in the bark of *Boswellia* trees is called frankincense [6]. The word frankincense is derived from the old French ''*franc encens'',* meaning "pure incense''[2]. The Arabic word for frankincense is *Luban,* denoting whiteness and inferring purity. The Hebrew name is *Levona;* the Greek, *Libanos* or *Libanotos;* the Latin, T*us* [2]; the Hindi*, Salai guggal* [7]*;* and the Amharic, *Etan* [8].

The genus *Boswellia* of the Burseraceae family includes more than 30 species of small trees that grow with the limited natural growing range[9-13], mainly in Arabia, on the eastern coast of Africa (Ethiopia, Somalia, Kenya, Eritrea), Yemen, Oman and in India [6, 14].

Taxonomical classification:

Division: Spermatophyta
Sub-division: Angiospermae
Tribe: Rosopsida
Sub-tribe: Rosidae
Super-class: Rutanae
Class: Anacardiales
Family: Burseraceae
Genus: *Boswellia* [15].

Table 1 shows representative *Boswellia* species and their geographical distribution [16-18]. Being a natural product, frankincense varies greatly in its ingredients. These variations can also be found in different varieties of the *Boswellia* tree. The tree can produce exudates in good quality only for three years. After this period, the quality of the collected resin decreases considerably. Therefore, the tree should be left to rest for some years after harvesting period [9, 19-21].

Frankincense is produced mainly by four species from different regions such as *B. serrata* from India, *B. sacra* (*Syn*. *B. carteri*) from East Africa, Middle East, and China, *B. frereana* from Northeast Africa (Somalia) and *B. papyrifera* from Ethiopia [10, 22, 23]. The four most-traded frankincense are produced in Oman, Yemen, Somalia, Ethiopia and Kenya [21]. The resin of *B. papyrifera* was considered as poorer quality than the product obtained from the Arabian and Somalian species [3, 12].

Boswellia serrata is one of the common plants used in Indian Ayurveda system of medicine. The resin is referred to as *Indian frankincense.* The Sanskrit name of the plant is “Gajabhakshya”. It is a deciduous middle-sized tree, which is mostly concentrated in the tropical region of Asia. In India, it occurs in dry hilly forests of Rajasthan, Madhya Pradesh, Gujarat, Bihar, Assam, Orrisa as well as central peninsular regions of Andhra Pradesh [21].

In Ethiopia *Boswellia* species are found in *Acacia-Commiphora* woodlands, wooded grasslands, and *Combretum-Terminalia* or broad-leaved deciduous woodlands. They grow at an altitudinal range of 950-1,800 m.

Table 1: *Boswellia* species and their geographical distribution

Boswellia Species	Geographical distribution	Ref.
B. ameero Balf.f.	Socotra, Yemen	[16-18]
B. boranensis Engl.		[16-18]
B. bricchettii Chiov.		[16-18]
B. bullata Thulin	Socotra, Yemen	[16-18]
B. carteri Birdw.	Somalia, *synonyms* with B. sacra	[16-18, 24]
B. chariensis Guillaumin	Synonyms with *B. papyrifera*	[16-18]
B. dalzielii Hutch.	Nigeria	[16-18]
B. dioscoridis Thulin	Socotra, Yemen	[16-18]
B. elegans Engl.	Himalaya, Tanzania	[16-18]
B. elongata Balf.f.	Socotra, Yemen	[16-18]
B. frereana Birdw.	Somalia	[16-18]
B. globosa Thulin	Somalia	[16-18]
B. hildebrandtii Engl.		[16-18]
B. holstii Engl.	synonyms of *B. neglecta*	[16-18]
B. madagascariensis Capuron	Madagascar	[16-18]
B. microphylla Chiov.	Ethiopia, Somalia, Kenya	[16-18, 25]
B. multifoliolata Engl.		[16-18]
B. nana Hepper	Socotra, Yemen	[16-18]
B. neglecta S.Moore	Ethiopia, Somalia, Kenya, Uganda	[16-18, 25]
B. occidentalis Engl.		[16-18]
B. odorata Hutch.		[16-18]
B. ogadensis Vollesen	Ethiopia	[16-18, 25]
B. ovalifoliolata N.P.Balakr.	India	[16-18]
B. papyrifera Hochst.	Ethiopia, Eritrea, Sudan	[16-18, 25]
B. pirottae Chiov.	Ethiopia	[16-18, 25]
B. popoviana Hepper	Socotra, Yemen	[16-18]
B. rivae Engl.	Ethiopia, Somalia, Kenya	[16-18, 25]
B. ruspoliana Engl.	synonms of *B. rivae*	[16-18]
B. sacra Flück.	Oman, Yemen, Somalia, Nubia	[16-18, 24]
B. serrata Roxb.	India	[16-18]
B. socotrana Balf.f.	Socotra, Yemen	[16-18]

The ecological distribution of species are confined to lowlands subjected to hot and arid conditions [26]. These vegetation types are found in the northern, north-western, western, eastern, south-eastern and south-western lowlands as well as along the major river gorges such as Blue Nile, Tekeze, Gibe and their tributaries [27-29].

Three types of frankincense products are recognized in Ethiopia, namely, the Tigray, Ogaden, and Borena types [26]. *B. papyrifera* is commonly known as Tigray type which is predominantly found in Tigray, Amhara and Benishangul-Gumuz regions [27, 29]. As reported by Asmare, the chemical composition of the *Boswellia* species found in Amhara region, Jawe woreda, Kebtele *kebele* was found to be different *B.* papyrifera [10].

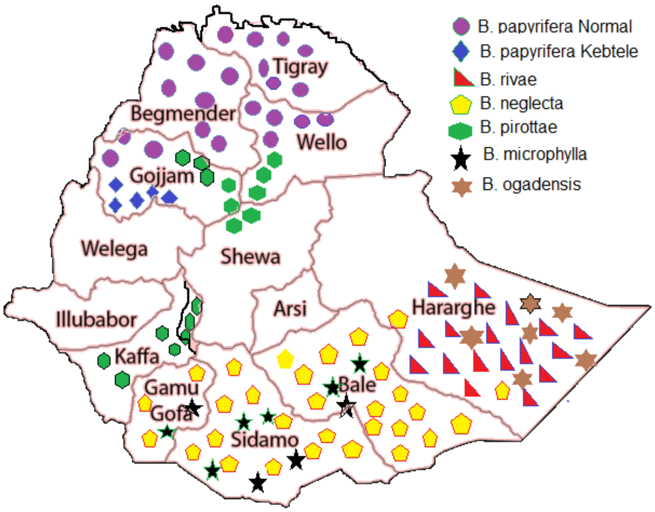


Figure 1: Distribution of *Boswellia* species in Ethiopia

The Ogaden type resin is obtained from *B. rivae* found in South-Eastern region*,* and Borena type resin is obtained from *B. neglecta* which is distributed in Southern areas of the country: Bale, Gamo-Gofa, Sidamo and also in Hararge. *B. pirottae* is found in Kefa, Wollo, Shewa, Gojam and Gonder. *B. microphylla* is found in Bale, Gamo-Gofa and Sidamo. *B. pirottae* and *B. microphylla* are the two rare types of endemic species to Ethiopia. The geographical distributions of *Boswellia* species in Ethiopia are shown in *Fig.*1 [30].

1.2. Cultural, traditional and pharmacological significance

Frankincense is used as incense, for religious services [1], in cultural ceremonies and as a traditional remedy for treating various diseases [7]. It has been also used as a fixative in perfumes, soaps, creams, lotions and detergents [11]. It is as also used in many countries of Africa, the Middle-East, China and India for treatment of chronic inflammatory diseases [9, 31], anti-arthritic, anti-proliferative and analgesic [32]. In traditional Chinese medicine (TCM), frankincense is commonly used as a remedy for improving blood circulation and for relieving pain in leprosy, gonorrhea and cancer [33]. Recently the use of olibanum is becoming popular specially in European countries for the treatment of chronic inflammatory problems [34][21].

Pharmacological activities of extracts of frankincense have been reported as anti-inflammatory, anti-analgesic, anti-arthritic, anti-cancer, immunomodulatory, leukotriene inhibition, hypolipidemic, hepatoprotective, hypoglycemic, anti-diarrhoeal, anti-microbial, and anti-asthmatic activity [7, 9, 21, 31, 35]. Various anti-cancer research studies and published data reported on safety on *B. serrata* can be used for the treatment of colon cancer, pancreatic cancer, brain cancer, leukemia, and prostate cancer [36].

Comparative studies on anti-inflammatory and anti-arthritic activities of frankincense in systemic and topical applications revealed that it is effective through both routes but the effect seems better via topical application in chronic arthritic inflammation [37]. Some of the branded ant-inflammatory formulations containing *B. serrata* available in the US and European markets are Boswellin®, Shallaki®, Niltan® and Rheumatic® [21].

The resin of the African *B. papyrifera* is widely used as the first choice as incense as well as to soothe the nerve during praying in Ethiopian, Syrian, Greece and Russian Orthodox Churches. It also enjoys a wide array of traditional uses as a human medicine and insect repellant. The bark is chewed to treat stomach disturbances and to prevent or quench thirst. The bark of *B. neglecta* is used as varnish after boiling. The frankincense is used as insect, rat and snake repellant and for the treatment of skin diseases. The resin of *B. rivae* is used to repel insects [8, 10]. In Eastern Ethiopia, the resins are also chewed to ease toothache, to treat lymphadenopathy [38].

1.3. Economic significance

Frankincense is the major commercial product obtained from *Boswellia* species and trade is restricted to the horn of Africa (Eritrea, Ethiopia, Kenya, Somalia, and Sudan), Middle East (Yemen, Oman) and India. Resins of *Boswellia* species are marketed with different trade names based on the origin of the resin. For example, *B. serrata* is traded as Indian type; *B. papyrifera* as Eritrean or Tigray type; *B. sacra (syn. B. carteri)* as Yemen or Oman type; *B. frereana* as Somali type; *B. neglecta* as Borena type and *B. rivae* as Ogaden type.

In Ethiopia gum resins (frankincense) contribute to local livelihoods in terms of both cash income and subsistence value [39]. Ethiopia is one of the largest suppliers of frankincense to the world market [40]. Gums and resins exported from Ethiopia include gum olibanum (frankincense) produced from *Boswellia* species, gum Arabic from *Acacia senegal*, gum talha from *A. seyal*, gum drepanolobium from *A. drepanolobium*, gum myrrh (*Kerbe* in Amharic) from *Commiphora myrrha*, and gum oppoponax (*Abeked* in Amharic) from *C. guidotti* [39]. The 2016 annual report of the Ethiopian Revenue and Custom Authority (ERCA) indicated that except for gum Arabic, the remaining gums and resins are exported as “natural gums, resins, gum-resins, natural oleorins/ balsams” of which frankincense covers more than 90% produced from *B. papyrifera*, *B. rivae* and *B. neglecta* [41].

The export data released by ERCA indicated that during 2016 fiscal year, it exported 2,224 tons of natural gums and resins to the value of more than 190 million Birr (about US$ 6.8 million) [41]. The exported volume remains much lower than the domestic market [29] but has been showing an increasing trend [29, 41]. In the period between 2002 and 2016, the average annual export was about 2879 tons. In this period, the most important export destinations of Ethiopian gums and resins 80% of were China (33.3%), United Arab Emirates (14.2%), Tunisia (9.1%), Germany (7.4%), Greece (6.9%), Yemen (3.1%), Guatemala (2.3%), India (1.5%), United States of America (1.2%), Saudi Arabia (1.0%) and Israel (0.2%) [41].

1.4. Chemical constituents of major species

According to Basar [42] the first investigation on the chemical composition of *Boswellia* resin was performed in 1788 by *Johann Ernst Baer* at the University of Erlangen. The composition of the essential oil and other contents changes from species to species depending on the climate, harvest conditions and geographical locations [11, 34, 43]. Frankincense contains 60-85% resins (mixtures of terpenes), 6-30% gums (mixture of polysaccharides) and 5-9% essential oil [20, 23, 32, 44, 45]. The resin portion is mainly composed of pentacyclic triterpenes of which boswellic acids are the biologically constituents [31, 46] and tetracyclic compounds [15].

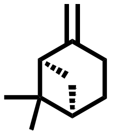
1.4.1. Essential oils

The constituents of the essential oils of *Boswellia* species were first investigated by J. Stenhouse in 1840 [47]. Stenhouse identified fourteen monoterpenoid constituents depending on the origin of the resins [42]. The yield of the volatile oil of frankincense obtained by hydro-distillation of the finely powdered oleogum resin derived from *Boswellia* species was reported in several articles [15, 23, 44, 46, 48-50]. Such studies revealed the presence of monoterpenes, sesquiterpenes and diterpenes [6, 23, 42, 46], *Fig.* 2. Monoterpenes found in *B. sacra* (*syn. B. carteri), B. serrata*, *B. papyrifera*, *B. frereana*, *B. neglecta*, *B. rivae* and *B. pirottae* have been reported in the literature [10, 22, 23, 31, 33, 34, 42, 51-55] as shown in Table 2.

Table 2: Monoterpenes reported from *Boswellia* species

Non-oxygenated monoterpenes
2-*β*-pinene (**1**), *α*-thujene (**2**), E-β-ocimene (**3**), 2,4(10)-thujadiene (**4**), camphene (**5**), sabinene (**6**), 1-*β*-pinene (**7**), myrcene (**8**), *α*-pinene (**9**), 2-carene (**10**) limonene (**11**), Z-*β*-ocimene(**12**), γ-terpinene (**13**), terpinolene (**14**), *p*-cymene (**15**), 1,4-cyclohexadiene (**16**), 1,3,6-trimethylene-cycloheptane (**20**), α-phellandrene (**35**), *β*-phellandrene (**36**), *δ*-3-carene (**46**), *o*-cymene (**47**), *m*-cymene (**48**), 2,5-dimethylstyrene (**64**), *trans*-sabinol (**80**)
Oxygenated monoterpenes
Perillene (**17**), isopentyl-2-methyl butanoate (**18**), isomyl valerate (**19**), *β*-thujone

(**21**), *α*-campholene aldehyde (**22**), allo-ocimene (**23**), trans-pinocarveol (**24**), *p*-mentha-1,5-dien-8-ol (**25**), 4-terpineol (**26**), sabinyl acetate (**27**), myrtenal (**28**), *α*-terpineol (**29**), α-phellandrene epoxide (**30**), verbenone (**31**), *trans-(+)*-carveol (**32**), carvone (**33**), bornyl acetate(**34**), methyleugenol (**37**), *cis*-verbenol (**38**), *trans*-verbenol (**39**), 1,8-cineole (**40**), α-pinene oxide (**41**), *p*-mentha-6,8-dien-2-one (**42**), neryl acetate (**43**), methylchavicol (**44**), linalool (**45**), *o*-methylanisol (**49**), *cis*-1,2-limonen oxide (**50**), *trans*-1,2-limonene oxide (**51**), *trans*-sabinene hydrate (**52**), chrysanthenone (**53**), linalyl acetate (**54**), umbellulol (**55**), *trans*-pinocarveol (**56**), carvotanacetone (**57**), *cis*-1,2-epoxy-terpin-4-ol (**58**), *p*-mentha-1,5-diene-7-ol (**59**), *p*-cymen-8-ol (**60**), *m*-cymen-8-ol (**61**), thymol (**62**), carvacrol (**63**), pinocamphone (**65**), *trans*-dihydrocarvone (**66**), sabinaketone (**67**), *cis*-p-mentha-2,8-diene-1-ol (**68**), *trans-p*-mentha-1(7),8-diene-2-ol (**69**), *cis-p*-mentha-1(7),8-diene-2-ol (**70**), myrtenol (**71**), piperitenone (**72**), borneol (**73**), verbenone (**74**), *β*-myrcene (**75**), nerol (**76**), camphor (**77**), *trans*-p-menth-2-en-1-ol (**78**), terpinyl acetate (**79**), *cis*-sabinol (**81**), *cis*-sabinene hydrate (**82**), pinocarvone (**83**), *trans*-carveol (**84**), *cis*-carveol (**85**), *cis*-p-menth-2-en-1-ol (**86**)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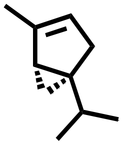
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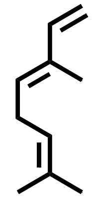
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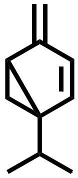
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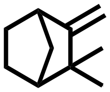
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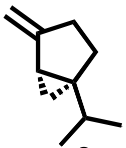
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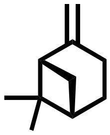
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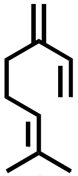
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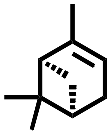
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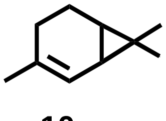
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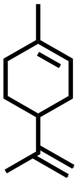
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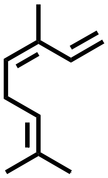
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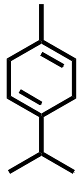
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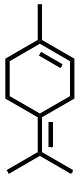
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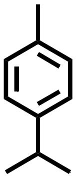
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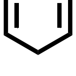
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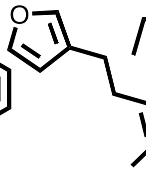
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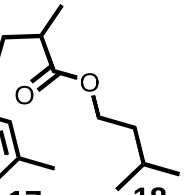
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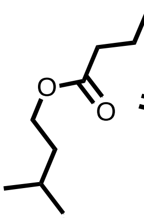
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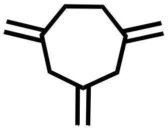
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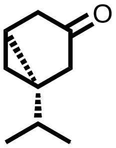
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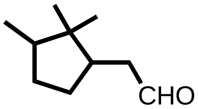
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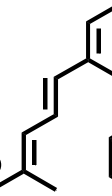
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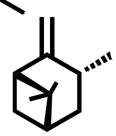
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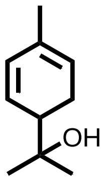
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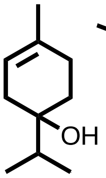
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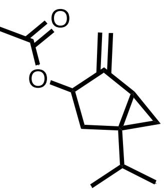
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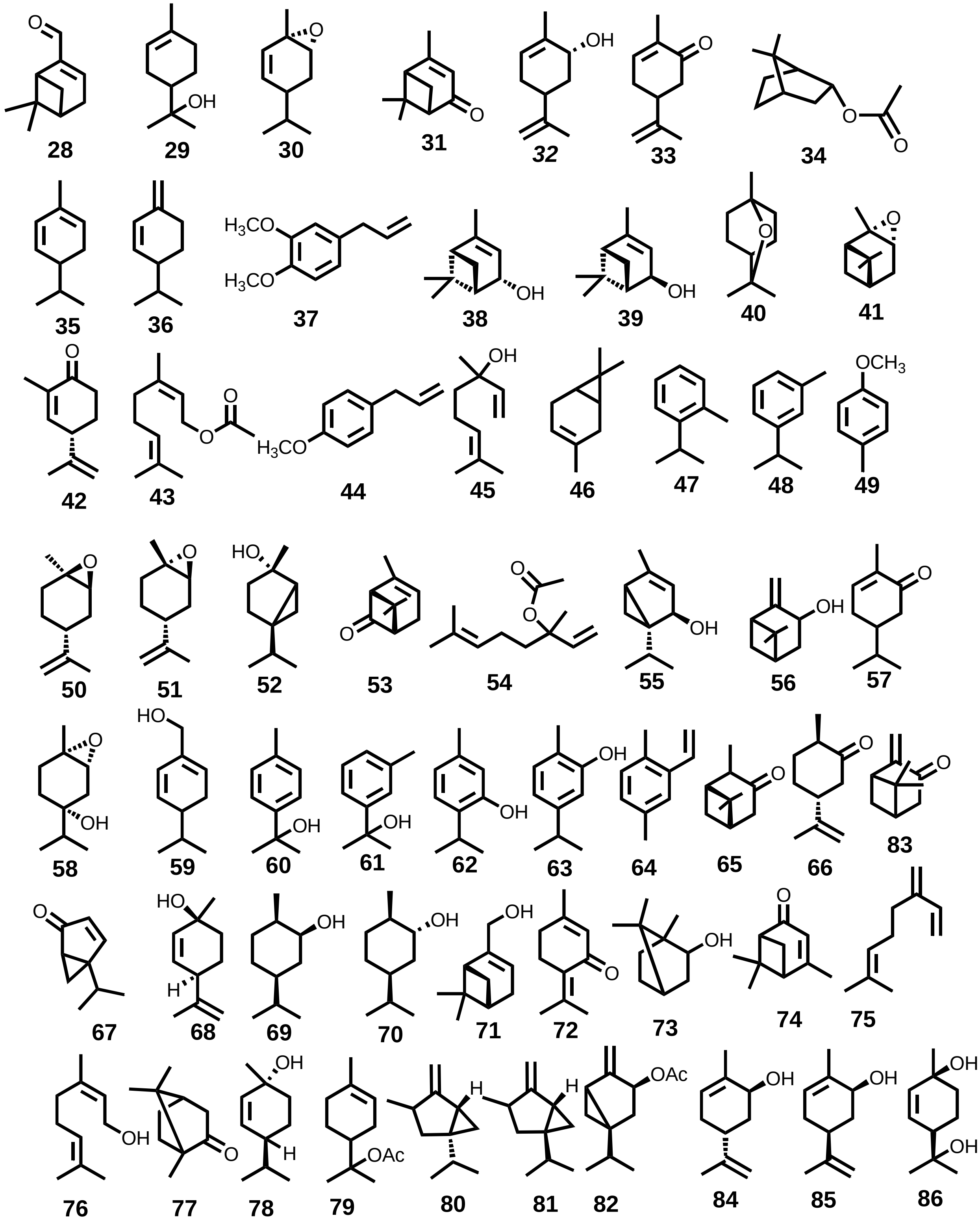


Figure 2: Structures of monoterpenes in the resin of *Boswellia* species

Sesquiterpenes and diterpenes are also the main components of essential oil of *Boswellia* resins, Fig 3 and 4. The major sesquiterpenes and diterpenes are presented in Table 3. Compounds other than terpenes are *n*-octyl acetate, *n*-octanol, *n*-hexyl acetate, decanol, decyl acetate, geranyl acetate, dodecanol, hexyl hexanoate, octyl formate [15, 54].

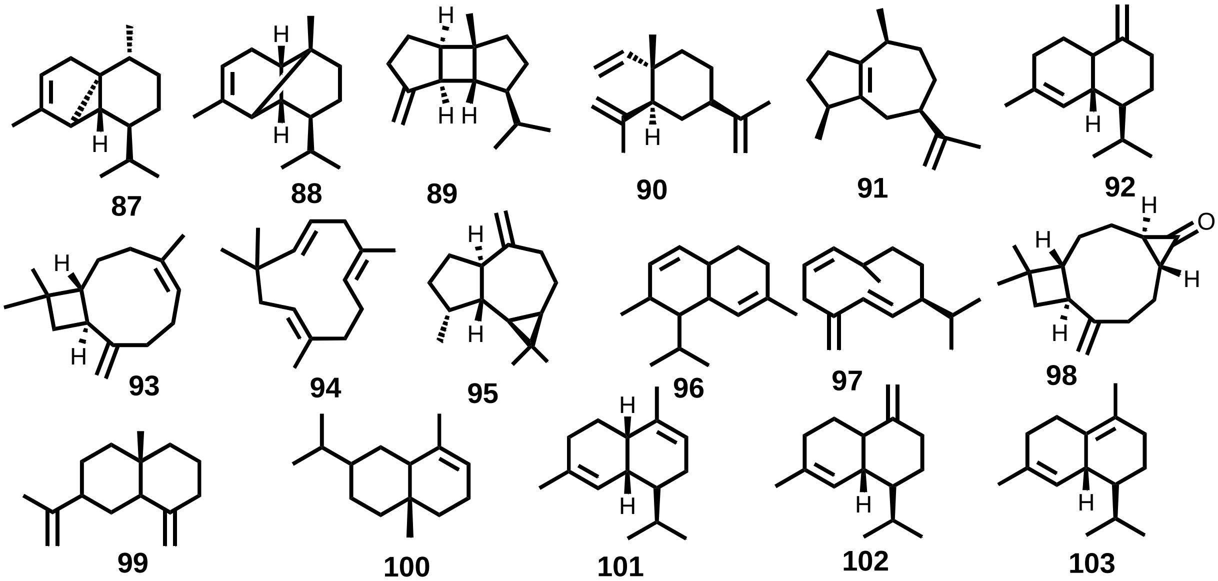


Figure 3: Structures of diterpenes in the resin of *Boswellia* species

Table 3: Sesqui- and diterpenes reported from *Boswellia* species

Terpenes	Name of compounds
Sesquiterpenes [23]	α-cubebene (**87**), α-copaene (**88**), ß-bourbonene (**89**), β-elemene (**90**), α-gurjunene (**91**), γ-muurolene (**92**), E-aryophyllene (**93**), α-humulene (**94**), alloaromadendrene (**95**), α-amorphene (**96**), germacrene D (**97**), caryophyllene oxide (**98**), β-selinene (**99**), α-selinene (**100**), α-muurolene (**101**), γ-cadinene (**102**), δ-cadinene (**103**)
Diterpenes [10, 42, 56]	verticill-4(20),7,11-triene (**104**), cembrene (**105**), cembrene A (**106**), cembrene C (**107**), serratol (**108**), incensole (**109**), *iso*-incensole oxide (**110**), incensole acetate (**111**), incensole oxide (**112**), *iso*-incensole (**113**), *iso*-incensole acetate (**114**), *iso*-incensole acetate oxide (**115**), *m*-camphorene (**116**), *p*-camphorene (**117**), *α*-phellanderene dimer (**118**), cneorubenol (**119**), *iso*-serratol (**120**)

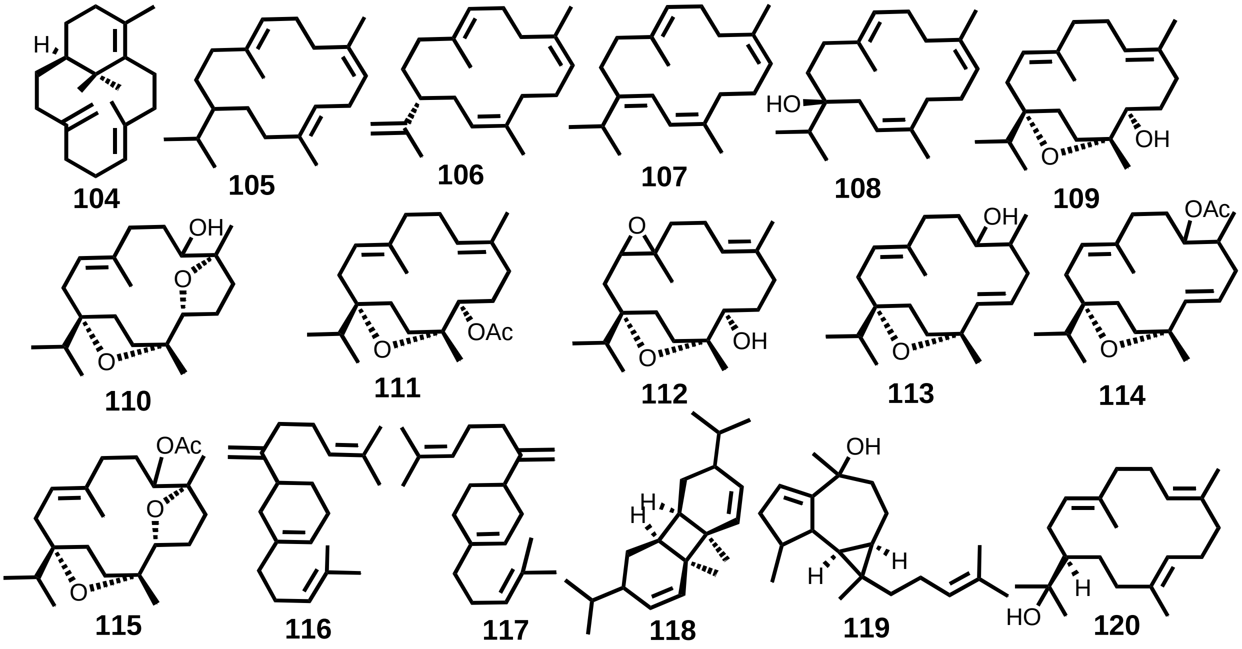


Figure 4: Structures of sesquiterpenes in the resin of *Boswellia* species

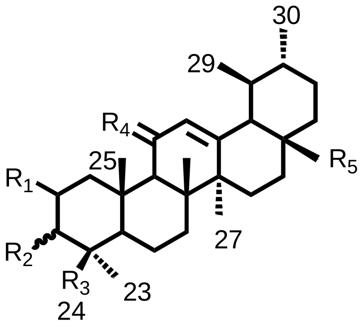
1.4.2. Triterpenes

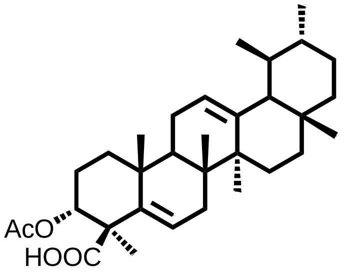
The presence of boswellic acid ($C_{32}H_{52}O_4$) in olibanum was reported for the first time in 1898 by Tschirch and Halbey [57]. Attempts were made to isolate resin aids with α- and β-amyrin like skeleton by Winterstein and Stein in 1932 [58]

More than 60 triterpenoids with ursane, oleanane, lupane, tirucallane, dammarane, euphane, robulic and canaric triterpene skeletons were reported from major *Boswellia* species (*Fig. 5a* and *5b,* [20]). The first three belong to the pentacyclic triterpenes class, whereas, the remaining are classified as tetracyclic triterpenes. *Boswellia* species are source of triterpinoids [20]. Table 4 gives the ursane- and oleanane-type triterpenoids, whereas, Table 5 gives lupane-, tirucallane-, dammarane-, euphane-, roburic- and canaric-type triterpenoids isolated from *Boswellia* species.

Table 4: Ursane- and oleanane-type triterpinoids

Ursane-type triterpenoids	Source	References
β-Boswellic acid (β-BA, **121**), 3-acetyl-β-BA (**122**), 11-keto-β-BA (**123**), 3-acetyl-11-keto-*β*-BA (AKβBA, **124**)	*B. serrata* *B. sacra*	[3, 31, 59-62]
urs-12-en-3,11-diketone (**125**), 2*α*,3*α*-dihydroxy-urs-12-en-24-oic acid (**127**), urs-12-en-3α,24-diol (**128**), 3*α*-acetyl-11-hydroxy-BA (**132**), urs-12-ene-3-β-24-diol (**134**), Urs-12-ene-3-α-24-diol (**135**)	*B. serrata*	[20, 63-66]
3α-acetyl-11*α*-methoxy-*β*-BA (**126**), 3β-Hydroxy-11-oxours-12-ene (**138**), 11β-Hydroxy-3-oxours-12-ene (**139**), 3α-acetylurs-5:12-dien-24-oic acid (**140**), 3*α*-acetyl-9,11-dehydro-*β*-BA (**141**), 9,11-dehydro-*β*-BA (**142**), 18H*α*, 3*β*,20*β*-ursanediol (**143**), 18Hα, 3β,20β-ursanediol (**144**), 3β,20S-dihydroxy taraxastane (**145**)	*B. sacra*	[62, 67-69]
α-amyrone (**129**), 3-epi-*α*-amyrin (**130**), α-amyrin (**131**), 3*α*-acetyl-28-hydroxy-11-oxours-12-en-24-oic acid (**133**)	*B. sacra,* *B. serrata* *B. neglecta*	[20,57[70-72]
methyl 3α-acetyl-11α-methoxy-urs-12-en-24-oate (**136**) and 3-oxo-24-acetyl-11α-hydroxy-urs-12-ene (**137**)	*B. rivae*	[73]
Oleanane-type triterpenoids		
α-Boswellic acid (**146**), 3*α*-acetyl *α*-BA (**147**), *β*-amyrone (**148**), 3-epi-*β*-amyrin (**149**), *β*-amyrin (**150**)	*B. sacra,* *B. serrata*	[31, 60, 74, 75]
3*α*,24-dihydroxy-olean-12-ene (**151**), 9,11-dehydro- *α*-BA (**153**), 3*α*-acetyl-9,11-dehydro-α-BA (**154**)	*B. serrata*	[64, 74, 76, 77]
olibanumol E (**152**: 3*α*-hydroxy-11-methoxyl-olean-12-ene)	*B. sacra*	[78]





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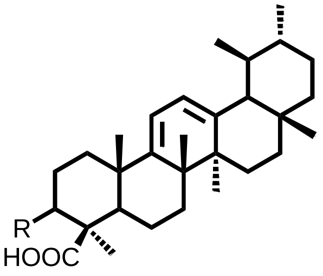
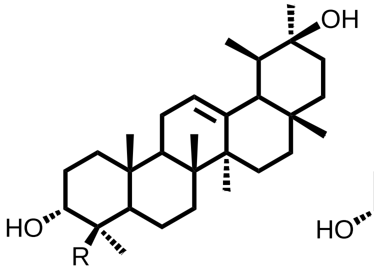


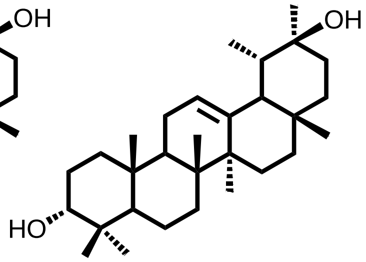
141 **142**

R α-OAc α-OH



143 **144**

R COOH CH$_3$



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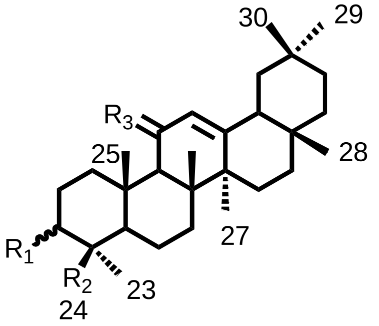
	R$_1$	R$_2$	R$_3$	R$_4$	R$_5$
121	H	α-OH	COOH	H,H	CH$_3$
122	H	α-OAc	COOH	H,H	CH$_3$
123	H	α-OH	COOH	O	CH$_3$
124	H	α-OAc	COOH	O	CH$_3$
125	H	=O	CH$_3$	O	CH$_3$
126	H	α-OAc	CO$_2$H	α-OCH$_3$	CH3
127	α-OH	α-OH	COOH	H, H	CH$_3$
128	H	OH	CH$_2$OH	H, H	CH$_3$
129	H	= O	CH$_3$	H, H	CH$_3$
130	H	α-OH	CH$_3$	H, H	CH$_3$
131	H	β-OH	CH$_3$	H, H	CH$_3$
132	H	-OAc	COOH	H, H	CH$_3$
133	H	α-OAc	COOH	= O	CH$_2$OH
134	H	α-OH	CH$_2$OH	H, H	CH$_3$
135	H	β-OH	CH$_2$OH	H, H	CH$_3$
136	H	α-OAc	CO$_2$CH$_3$	OCH$_3$, H	CH$_3$
137	H	= O	CH$_2$Ac	OH, H	CH$_3$
138	H	-OH	CH$_3$	= O	CH$_3$
139	H	= O	CH$_3$	-OH	CH$_3$

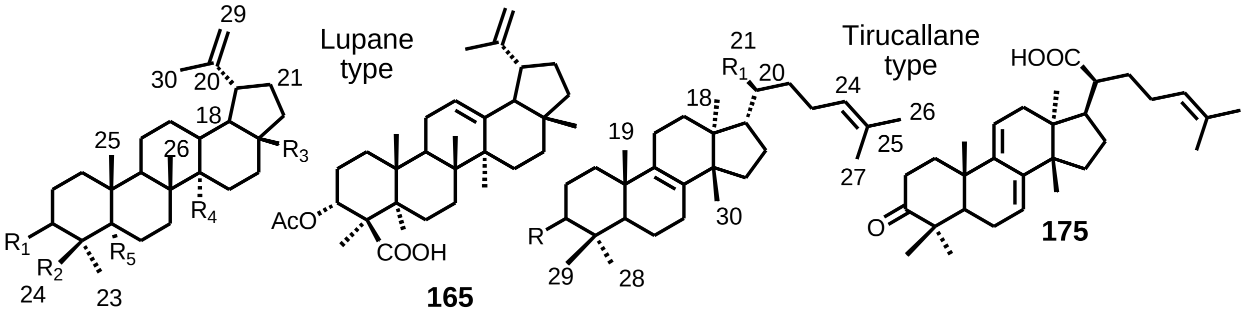


	R$_1$	R$_2$	R$_3$
146	α-OH	COOH	H,H
147	α-OAc	COOH	H,H
148	= O	CH$_3$	H, H
149	α-OH	CH$_3$	H, H
150	β-OH	CH$_3$	H, H
151	α-OH	CH$_2$OH	H, H
152	α-OH	CH$_3$	α-OCH$_3$, H
153	a-OAc	COOH	H, H
154	α-OH	COOH	H, H

Figure 5a: Ursane- and oleanane-type triterpenes isolated from the *Boswellia* species

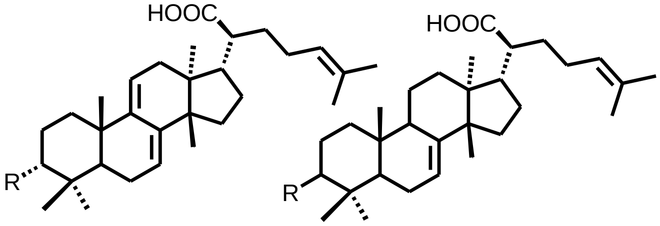
Table 5: Lupane-, tirucallane-, dammarane-, euphane-, roburic- and canaric-type triterpenoids

Lupane-type triterpenoids	Sources	Ref.
Lupeolic acid (**155**), acetyl-lupeolic acid (**156**), 3α-acetyl-28-hydroxy-lupeolic acid (**160**), Epilupeol acetate (**163**), 3*α*, 5α-dihydroxy-lup-20(29)-en-24-oic acid (**164**), 3α-acetoxylup-12:20(29)-dien-24-oic acids (**165**)	*B. sacra*	[68, 77-79]
lupenone (**157**), epi- lupeol (**158**), lupeol (**159**)	*B. frereana* *B. sacra*	[31, 60]
3*α*-acetyl-27-hydroxy-lupeolic acid (**161**), methyl-3*α*-acetyl-27-hydroxy-lupeolic acid (**162**)	*B. papyrifera*	[80]
Tirucallane-type triterpenoids		
α-elemolic acid (**165**), elemonic acid (**166**), *β*-elemolic acid (**167**), 3*β*-acetyl-tirucallic acid (**168**)*,* 3*α*-acetyl-tirucallic acid (**169**)	*B. sacra* *B. serrata*	[31]
3β-hydroxy-tirucall-8,24-diene (**170**), 3*α*-hydroxy-tir-7,24-dien-21-oic acid (**173**), 3*α*-acetyl-tirucallic acid (**174**), 3-oxo-tir-7,9(11),24-trien-21-oic acid (**171**), 3-oxotirucalla-7,9(11),24-trien-21-oic acid (**172**), 3-oxolanosta-7,9(11),24-trien-21-oic acid (**175**) .	*B. sacra*	[24, 69]
Dammarane and Euphane-type triterpenes		
3*β*-acetyl-16(S),20(R)-dihydroxy-dammar-24-ene (**176**), 3*β*,20*(S)*-dihydroxy-dammar-24-ene (**177**), 3*β*-acetyl-20(S)-hydroxy-dammar-24-ene (**178**), 20*(S)*-protopanaxadiol (**179**)	*B. frereana*	[81]
20,22-epoxyeupha-24-ene-3-one (**180**)	*B. serrata*	[20]
Roburic and Canaric-type triterpenes		
Roburic acid (**181**), 11-keto-roburic acid (**182**), 4(23)-dihydro-roburic acid (**183**), 4(23)-dihydro-11-keto-roburic acid (**184**)	*B. sacra*	[24]
Canaric acid (**185**)	*B. neglecta*	[8, 70, 72]



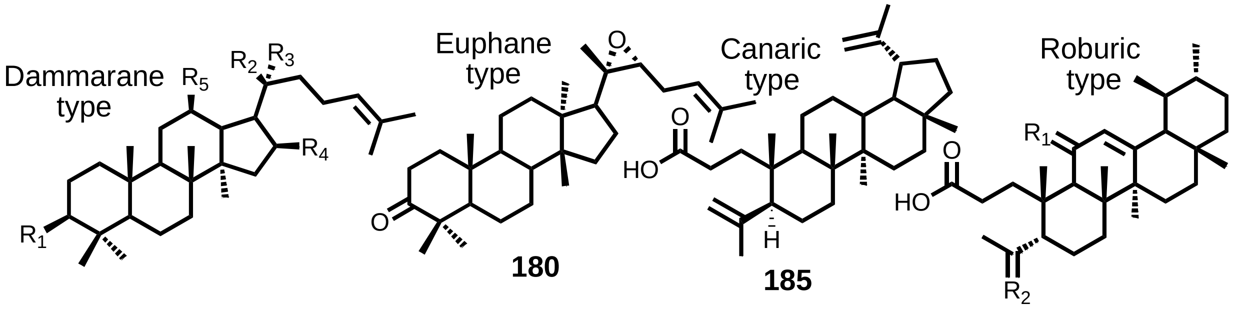
	R1	R2	R3	R4	R5
155	α-OH	COOH	CH_3	CH_3	H
156	α-OAc	COOH	CH_3	CH_3	H
157	= O	CH_3	CH_3	CH_3	H
158	α-OH	CH_3	CH_3	CH_3	H
159	β-OH	CH_3	CH_3	CH_3	H
160	α-OAc	COOH	CH_2OH	CH_3	H
161	α-OAc	COOH	CH_3	CH_2OH	H
162	α-OAc	$COOCH_3$	CH_3	CH_2OH	H
163	α-OAc	CH_3	CH_3	CH_3	H
164	a-OH	CH_3	CH_3	CH_3	OH

	165	**166**	**167**	**168**	**169**	**170**
R	α-OH	=O	β-OH	β-OAc	α-OAc	β-OH
R1	CO_2H	CO_2H	CO_2H	CO_2H	CO_2H	CH_3



	171	**172**
R	α-OH	=O

	173	**174**
R	α-OH	α-OAc



	R1	R2	R3	R4	R5
176	OAc	CH_3	OH	OH	H
177	OH	OH	CH_3	H	H
178	OAc	OH	CH_3	H	H
179	OH	OH	CH_3	H	OH

	181	**182**	**183**	**183**
R1	H,H	= O	H, H	= O
R2	CH_2	CH_2	CH_3	CH_3

Figure 5b. Lupane-, tirucallane, dammarane-, euphane-, canaric- and robulic-type triterpenes

Chemotaxonomy classification of *Boswellia* species was found to be better than other physical parameters to distinguish among species. To this end, different efforts have been made to develop a simple method to distinguish resins obtained from *Boswellia* species. However, the problem still persists to identify one species from the other, especially in *B. papyrifera* and *B. sacra* (*Syn*. *B. carteri).* Several literature reports claimed that n-octyl acetate (**89**), incensole acetate (**111**), and incensole (**109**) as the main components of the essential oil of *B. sacra* [7, 14, 23, 38, 44, 123,[55]. Nevertheless, these marker compounds are the major chemical components of *B. papyrifera* [45, 46, 82]. In 2005, Basar [42] compared five resin samples using both GC-MS and TLC and he reported these compounds as the major components of *B. sacra.* In 2012, Paul reported a TLC method for the identification of resins of *B. serrata, B. papyrifera and B. sacra* and he demonstrated that n-octyl acetate (**189**) and incensole acetate (**111**) were the main constituents of *B. papyrifera* [48]*.* The same year Woolley *et al*. reported a method for the chemical differentiation of *B. sacra and B. carteri* essential oils by GC and chiral GC-MS [83]. Neither **111** nor **189** were detected in both resins.

1.5. Biological activities of compounds isolated from frankincense

Triterpenoids from the genus *Boswellia* exhibit medicinal and pharmacological activities against various health-related conditions, in particular to control spread of inflammation, microbial infection and cancer [20, 84]. Active principles of a gum resin from *Boswellia* species have recently gained attention as chemo-preventive compounds due to their ability to target key enzymes such as 5- lipoxygenases (5-LOX), cyclooxygenases (COX) and nuclear factor-kappa B (NF-κB) [85]. COX-2 and 5-L OX, two enzymes involved in the oxygenation of arachidonic acid (AA), are up-regulated in the central nervous system during and are associated with various aging-related brain pathologies [20, 84].

1.5.1. Anti-inflammatory activities

The anti-inflammatory activities of the triterpenoids are largely ascribed to the inhibition of 5-LOX, nitric oxide synthase (iNOS), COX-2 and NF-κ B activities. Triterpenoids isolated from *Boswellia* species showed an alleviative effect on acetaminophene-induced hepatic injury by attenuated hepatic inflammatory infiltration [35, 86].

The ursane-type [63, 87-98], oleanane-type [20, 87, 89], lupane-type [87, 99-101] and tirucallane-type [102, 103] triterpenoids which were isolated from resins of *Boswellia* species have been reported as an anti-inflammatory compounds. In addition to triterpenoids, the most known diterpenes serratol (**108**), incensole (**109**) and incensole acetate (**110**) showed anti-inflammatory activity [14, 104].

1.5.2. Anti-cancer and anti-tumor

Cancer is the uncontrolled growth of abnormal cells anywhere in a body. These abnormal cells are termed cancer cells, malignant cells, or tumor cells. These cells can infiltrate normal body tissues [36]. Currently, chemotherapy, radiation therapy, immunotherapy, photodynamic therapy, hormonal therapy, and surgery are used for cancer treatment. The therapeutic success rate for cancer can be improved by use of natural products [36].

Resinous triterpenoids, including boswellic acids, have anti-cancer activity as a result of their anti-proliferative and pro-apoptotic properties [49, 66, 105]. Cell proliferation is the process that results in an increase of the number of cells whereas apoptosis is a process of programmed cell death. Targets for anti-cancer compounds are cytochrome P450 [106, 107], nuclear factor-kappa B (NF-κB) [108-110] and topoisomerase IIα [111]. The anti-cancer activities of ursane-type [67, 94, 95, 105, 112-114], oleanane-type [64, 76, 115], lupane-type [20, 67, 116, 117] and tirucallane-type triterpenoids [67] have been reported.

1.5.3. Apoptosis-regulating activities

Apoptosis (programmed cell death) is a form of cell death that is morphologically and biochemically distinct from necrosis (non-programmed cell death) [118]. Previous studies have indicated that apoptosis provides a critical regulatory mechanism in inflammatory processes [119] and cancer. Cell apoptosis activities of boswellic acids have been reported [94, 120, 121].

In addition to the above activities, compounds isolated from resins of *Boswellia* species exhibits anti-bacterial and anti-fungal activities [49, 82, 122, 123], inhibitory activity against prolyl endopeptidase, increase the polymerization rate of tubulin [124], anti-elastase activity [125], inhibitory effects on a purified HIV-1 reverse transcriptase [126], inhibit protein tyrosine phosphate [127] and immunomodulatory properties [128] have been reported. In laboratory studies, essential oil of *B. papyrifera* was shown to have anti-molluscicidal properties on two species of snails [38, 129] and anti-plasmodial activity [104].

1.6. Aims of this dissertation

- Chemical study of resin of *B. pirottae* which was not investigated before.
- Comparative study of two distinct collections of *B. papyrifera* resin collected from Kebtele and other parts of Ethiopia [10].
- Isolating marker compounds from resins of *B. papyrifera* Kebtele*, B. papyrifera, B. sacra, B. serrata, B. frereana, B. pirottae, B. neglecta* and *B. rivae* by qualitative TLC and GC-MS methods in order to distinguish one species from the other.
- Quantification of the most active anti-inflammatory compounds, 3-acetyl-11-keto-β-boswellic acid (AKBA) and 11-keto-β-boswellic acid (KBA), found in the resin of *B. papyrifera* and *B. papyrifera* Kebtele in comparison to the well-known *B. sacra* and *B. serrata.*

2. Results and discussion

2.1. Specimen collection and identification

The report on the Burseraceae of Ethiopia by Vollesen revealed the occurrence of six *Boswellia* species in Ethiopia, namely, *B. papyrifera*, *B. pirottae*, *B. rivae*, *B. neglecta, B. ogadensis* and *B. microphylla* [25]. The resins of indigenous *Boswellia* species analyzed in this work were collected by Aman Dekebo and Asmare Melese in 2002 and 2007. The specimens were identified by Professor Sebsibe Demisew and Ato Melaku Wondafrash and deposited at the National Herbarium, Addis Ababa University, with voucher number *B. papyrifera* (072807), *B. neglecta* (072813), *B. rivae* (072808) and *B. pirottae* (072815) [8]. *B. ogadensis* and *B. microphylla* are not included in this study.

In the course of this work, field studies were undertaken to collect resins and specimens. Specimens of *B. papyrifera* were collected from Dehana (Sekota) and Gojjam (Debre-Markos), whereas, *B. pirottae* was collected from Tis-Abay (Gojjam) and Gibe Valley (Kefa). The resin collected from Sekota was identified as *B. papyrifera* (Sisay-01-2016), whereas, resins collected from Gibe and Abay valleys were both identified as *B. pirottae* (Sisay-01-2017 and Sisay-01-2017, respectively), by the National Herbarium, AAU. Moreover, resins were compared with the previous collection (Aman's collection) using TLC.

The resin of *B. papyrifera* (Kebtele) was collected by Asmare Melese in 2007 from Amhara Region, Agew-Awi Zone. The collection site was Jawe Woreda which is located west of Dangila (563 km from Addis Ababa). Barbas, Denbih, Kokolag, Kubaba, Zerzer, Zenzen, Mariamweha, and Ayshal are some harvesting sites in *Kebtele*, which is one of the *Kebele* in Jawe Woreda [10]. The resins of *B. frereana* and *B. serrata* were obtained from Somalia and India, respectively. *B. sacra* and *B. carteri* are synonyms and their similarity was confirmed by TLC analysis in addition to literature review and information from known botanical gardens [16-18]. The resin of *B. sacra* was obtained from Oman, Yemen and Somalia. In this thesis, the sample obtained from Somalia (which is known as *B. carteri*) was used for analysis. All fresh samples were compared with previously authenticated samples using TLC method.

2.2. Isolation and characterization of compounds from *Boswellia* resins

Frankincense is rich in water-insoluble (resin) and water-soluble (gum) constituents. In this work, the selection of suitable solvent for extraction of frankincense obtained from different *Boswellia* species was performed by dissolving 5 g of powdered resin in 10 mL of hexane, chloroform, ethanol and water. The resin of *B. papyrifera*, *B. papyrifera* Kebtele collection, *B. sacra* (syn. *B. carteri*) and *B. serrata* were found to be highly soluble in EtOH due to the presence of polar compounds, whereas, resins of *B. frereana*, *B. pirottae*, *B. neglecta* and *B. rivae* are highly soluble in hexane. However, for consistency extraction solvent in this thesis was EtOH.

The crude extract was obtained from extraction of 20 g of the resin with EtOH and subjected to a sequential base and acid extraction (Section 4.2.3) to afforded neutral and acidic fractions. After each treatment, the organic layer was separated using EtOAc and the percentage yields of neutral and acid fraction were calculated on a dry weight basis. As shown in Table 6, the acid contents of *B. papyrifera* Kebtele resin and *B. papyrifera* resin were higher than other resins investigated here.

This work was focused on the chemical investigation of *B. papyrifera, B. papyrifera* Kebtele and *B. pirottae* resins. However, in the resins of *B. frereana*, *B. sacra*, *B. neglecta* and *B. rivae* it focused on isolating marker compounds that distinguished one species from the other. The marker compounds of each resin were found in the neutral fractions, whereas, acid fractions were rich in triterpenes terpenes.

Table 6: Percent yields of neutral and acid fractions

***Boswellia* species**	**Neutral fractions**			**Acid fractions**		
	abbr.	amount(g)	(%)	*abbr.*	amount(g)	(%)
B. papyrifera	BpRn	11	55	BpRa	6.0	30
*B. papyrifera (*Kebtele)	BpKRn	12	60	BpKRa	7.0	35
B. sacra (Syn: B. carteri)	BsaRn	11	55	BsaRa	4.0	20
B. serrata	BsRn	14	70	BsRa	5.0	25
B. frereana	BfRn	18	90	BfRa	1.5	7.5
B. pirottae	BpiRn	17	85	BpiRa	1.0	5.0
B. neglecta	*BnRn*	15	75	BnRa	1.5	7.5
B. rivae	BrRn	16	80	BrRa	3.0	15

2.2.1. Compounds isolated from *B. papyrifera* (Kebtele Collection)

B. papyrifera resin collected from B. papyrifera trees occurring in a locality called Kebtele in North-West Ethiopia showed significant difference in its chemical profile than *B. papyrifera* occurring in other parts of the country. The crude extract of the resin is shown on TLC (*Fig. 6, 13a* and *13b*). The neutral fraction (BpKRn, section 4.2.3) was chromatographed over silica gel eluting with petrol:EtOAc mixtures of increasing polarities to afford verticilla-4(20),7,11-triene (**104,** BPK1) and cneorubenol (**119,** BPK5). These two compounds were isolated for the first time from the same species by Asmare in 2007 [10]. Compound **119** is an unusual diterpene in the genus *Boswellia.*

Nine higher triterpene and one fatty acid compounds were isolated from the acid fraction (BpKRa, section 4.2.3), of which two tetracyclic triterpenes (tirucallic acids) and seven pentacyclic triterpenes (boswellic acids) were isolated. Compounds were identified as 11-eicosanoic acid (**186**, BPK6), 3α-acetyl-tirucall-8,24-dien-21-oic acid (ATA, **169**, BPK7) and 3-oxo-tirucall-8,24-dien-21-oic acid (3-OTA, **166**, BPK8), a mixture of 3α-acetyl-α-boswellic acid (AαBA, **147**, BPK9) and 3α-acetyl-β-boswellic acid (AβBA, **122**, BPK10), AKBA (**124**, BPK11), a mixture of α-BA (**146**, BPK13) and β-BA (**121**, BPK12), 11-OH-BA (**132**, BPK14) and KBA (**123**, BPK15).

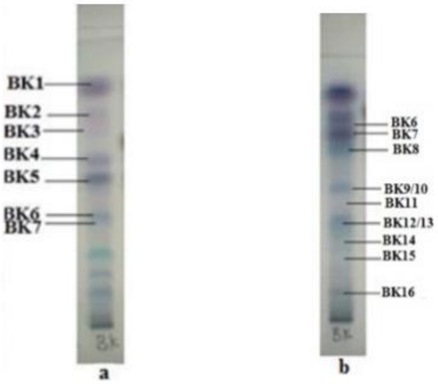
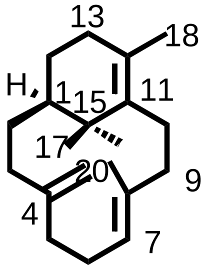


Figure 6: TLC of the EtOH extract of *B. papyrifera* Kebtele resin: a: neutral fraction (ss_1); b: acid fraction (ss_2)

Characterization of Verticilla-4(20),7,11-triene (104)

Verticilla-4(20),7,11-triene (**104**, BPK1) was obtained as a colorless viscous oil. It showed a strong violet spot on TLC (ss_1) at *Rf* = 0.74 after visualizing with vanillin/sulfuric acid. The IR spectrum (*KBr*) showed a strong absorption band at 2923 cm^{-1} due to aliphatic CH_3 and CH_2 groups, the band at 1441cm^{-1} is due to C-H bending. A weak absorption band at 3087 cm^{-1} indicated the presence of olefinic C-H. The absorption band at 1646 cm^{-1} is indicative of C=C stretching. The ^1H NMR spectrum (Appendix 1) of **104** in $CDCl_3$ showed four singlets at δ 0.96, 1.02, 1.6 and 1.7 due to methyl protons. Three olefinic proton signals were observed at δ 5.15 (1H, dd, *J* = 10.4, 3.4 Hz, H-7), 4.62 (dd, *J* = 3.6, 1.5 Hz, 1H, H-20) and 4.70 (1H, *s*, H-20). The two proton resonances which appeared at δ 4.62 and 4.70 were assigned to the geminal protons on an exocyclic double bond.

The ^{13}C NMR spectrum revealed 20 carbon atom signals as presented in Table 7 and Appendix 1. The DEPT-135 spectrum indicated the presence of four methyl, nine methylene, two methine and five quaternary carbon signals. The six carbon signals which appeared in the olefinic region were suggestive of the presence of three double bonds in the molecule of which signals at δ_C 108.2 and 153.6 were assigned for the exocyclic double bond. The above spectroscopic data were compared with those reported in the literature for the verticilla-4(20),7,11-triene and were found to be in a good agreement [10, 52]. Compound **104** was first from *B. carteri* as described by Basar [52] and from *B. papyrifera* as claimed by Paul [24]. Later, **104** was reported from *B. papyrifera* Kebtele [10] and *B. papyrifera* resins [24].



104

Characterization of cneoruben-10-ol (119)

Cneoruben-10-ol (**119**, BPK5**)**, $C_{20}H_{34}O$, was isolated as a yellowish viscous oil. Analysis by TLC (ss_1) showed a strong violet spot at *Rf* = 0.39 after dyeing with vanillin/H_2SO_4. The LC-MS data of **119** indicated molecular ion peak at *m/z* 289.2 (M^+) and a base peak at *m/z* 273.2 (M-OH). The IR spectrum showed a broad-band at 3429 cm^{-1} due to hydroxyl group and bands at 1373 cm^{-1} and 1237 cm^{-1} due to C-O stretching and O-H bending, respectively. The absorption band at 1656 cm^{-1} was indicative of C=C stretching.

Table 7: ^1H and ^{13}C NMR spectral data of **104** and **119**, and comparison with the data reported for verticilla-4(20),7,11-triene and cneorben-10-ol in the literature

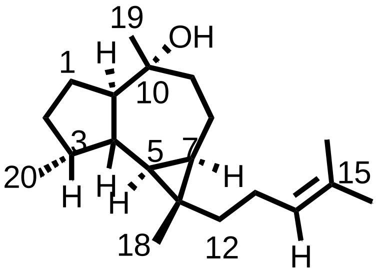
400 MHz, $CDCl_3$, δ in ppm, *mult*., *J* in Hz

C	**104**		Lit. [10]		**119**		Lit. [10]	
	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C
1	1.46-1.53	43.6	1.46-1.53	43.7	1.42, 1.77	25.8	1.40, 1.71	25.8
2	1.60-1.62, 2.0-2.1	31.6	1.65-1.69, 2.0-2.1	31.7	1.10, 1.50	29.2	1.08, 1.50	29.2
3	2.78 (*m*), 2.0-2.1	32.5	2.80(*td*), 2.0-2.1	32.5	1.78-1.87	38.8	1.78-1.91	38.8
4		153.6		153.7	1.69-1.77	39.2	1.68-1.76	39.2
5	2.04-2.07, 2.26-2.30	36.1	2.0-2.1, 2.24-2.30	36.2	0.12 (*t*)	22.2	0.00 (*t*)	22.2
6	2.16-2.23	29.6	2.0-2.1, 2.0-2.15	29.6		22.4		22.4
7	5.16(*dd*)	129.5	5.15(*dd*)	129.6	0.613(*ddd*)	27.3	0.458(*ddd*)	27.3
8		133.5		133.5	1.24, 1.42	18.8	1.20, 1.45	18.8
9	2.47(td),	39.2	2.45(tm)	39.3	1.38, 1.61	37.6	1.38, 1.60	37.6
10		25.7	1.40-1.47	25.7		74.6		74.7
11		136.4		136.5	1.61-1.69	58.2	1.60-1.70	58.3
12		127.5		127.6	0.79, 1.42	43.4	0.71, 1.40	43.4
13	2.03-2.07, 1.85-1.89	30.4	2.04-2.12, 1.80-1.90	30.4	1.96-2.01	25.3	1.95-2.00	25.3
14	2.1-2.16,	25.4	2.12-2.16,	25.4	5.01(*tm*)	125.0	4.95 (*tm*)	125.0

	2.20-2.30		2.20-2.30					
15		37.3		37.3		130.7		130.8
16	1.01	33.1	1.02(s)	33.2	1.58	17.6	1.46 (*s*)	17.6
17	0.99	26.6	0.96(s)	26.6	1.65	25.7	1.53 (*s*)	25.7
18	1.71	20.9	1.7(s)	20.9	0.889	13.3	0.858 (*s*)	13.4
19	1.64	16.7	1.6(s)	16.7	1. 01	32.1	1.01 (*s*)	32.2
20	4.70 (*s*), 4.64(*dd*)	108.2	4.70, 4.62	108.2	0.880	16.5	0.806 (*d*)	16.6

The ^{1}H NMR (Appendix 2) spectrum of **119** showed a total of five methyl proton signals at δ 0.88 (3H, *d*, *J* = 6.8 Hz, H-20), 0.86 (3H, *s*, H-18), 1.01(3H, *s*, H-19), 1.56 (3H, *s*,H-16), 1.65 (3H, *s*,H-17). The most up field signals at δ 0.12 (1H, *t*, *J* = 9.6 Hz, H-5) and at δ 0.62 (1H, *ddd*, *J* = 6.4, 7.4, 11.0 Hz, H-7) were a typical signals of protons on the cyclopropane ring. The downfield signal which resonated at δ 5.0 (1H, *t*, J = 7.2 Hz, H-14) was indicative of an olefinic proton.

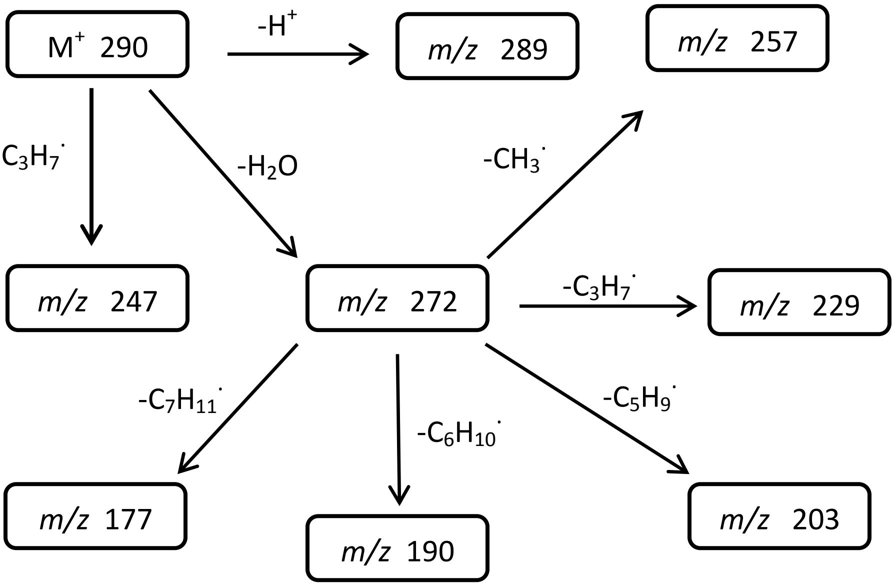
The ^{13}C NMR and DEPT-135 spectra (Appendix 2) indicated the presence of 20 carbon signals with five methyl, six methylene, six methine and three quaternary carbon signals. The two carbon signals at δ_C 125.0 and 130.7 were indicative of the presence of only one double bond in the molecule which was further confirmed by ^{1}H NMR (δ 5.01) and IR (1656 cm^{-1}) data. The deshielded methine carbon signal at δ 74.6 was due to the presence of OH group at C-10. Compound **119** was first isolated from *Boswellia* species by Asmare in 2007 [10]. Compound **119** was reported by Trautmann in 1980 as cneoruben-10-ol from *Cneorum tricoccon* [130] comparison of the above spectroscopic data with that reported by Asmare revealed a very close agreement (Table 7).



119

The EIMS spectrum of **119** revealed a molecular ion peak at *m/z* 290 (M^+) and the remaining mass fragments are shown in *Scheme 4.*

The biosynthesis of diterpenes containing cyclopropane ring from geranyl geranyl diphosphate was reported by Dulcie *et al.* [131].



Scheme 4: Proposed mass fragmentation of compound **119**

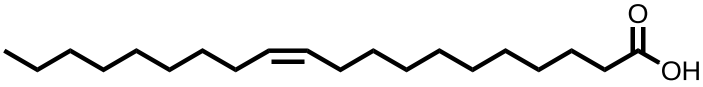
Characterization of 11-eicosanoic acid (186)

11-Eicosanoic acid (**186**, BPK6), was isolated as a white solid. Analysis by TLC (ss_1) showed a strong violet spot at *Rf* = 0.39 after dyeing with vanillin/H_2SO_4 reagent. The EIMS of **186** indicated a molecular ion peak at *m/z* 310 and base peak at *m/z* 254. The IR spectrum of **186** showed a weak broad absorption band at 3428 cm^{-1} due to the hydroxyl group of an acid. The presence of a strong band at 1704 cm^{-1} was due to the carbonyl carbon of an acid. A very weak band at 1621cm^{-1} can attributed to C=C bond stretching.

The ^1H NMR spectrum (Appendix 3) of **186** revealed the presence of a multiplet at δ 5.36 (2H, H-11, and H-12) due to two olefinic protons. The triplet at δ 0.89 (3H, *t*, *J* = 6.8

Hz, H-20) is a typical of terminal methyl protons of a fatty acid. A broad triplet at δ 2.36 (2H, *t*, *J* = 2.37 Hz, H-2) revealed the presence of the two methylene protons α to a carboxyl group. The multiplet which is resonated at δ 2.02-2.087 was indicative of methylene protons γ to the carboxyl group. The doublet of triplet at δ 1.63 (J = 12.0 and 6.8 Hz) was assigned to methylene protons adjacent to the double bonded carbons. The strong broad singlet at δ 1.27 was due to the remaining aliphatic methylene protons.

In the ^{13}C NMR the signal at δ 14.2 was a characteristic signal for a terminal methyl carbon of a fatty acid. The most downfield signal at δ_C 179.4 was also suggestive of a carboxyl functional group which was confirmed by a strong band at 1704 cm^{-1} in the IR spectrum. Two methine carbon signals at δ_C 129.7 and 129.8 indicated the presence of one double bond. The DEPT-135 spectrum showed ten intense methylene carbon signals at δ_C 22.71, 24.71, 29.09, 29.27, 29.35, 29.46, 29.6, 29.67, 31.95 and 33.99 and two methine carbon signals at δ_C 129.7 and 129.8. The absence of aliphatic methine carbon signals confirmed that the fatty acid has an unsubstituted long chain skeleton. Based on the above spectroscopic data compound 186 was identified as 11-eicosanoic acid or gondoic acid. It is a monounsaturated fatty acid, which is one of the omega-9 fatty acids, represented as ω-9 or n-9.



186

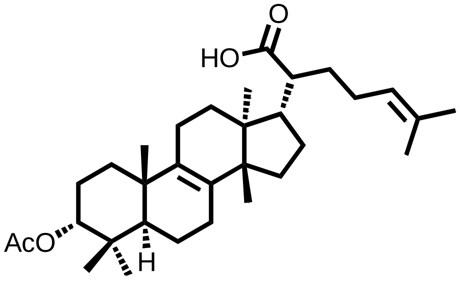
Characterization of 3*α*-Acetyl-tirucallic acid (169)

3*α*-Acetyl-tirucallic acid (**169**, BPK7) was obtained as white powder. On TLC (ss_2) a violet spot was observed at *Rf* = 0.60 with vanillin/H_2SO_4 reagent. The IR spectrum displayed an absorption band at 3376 cm^{-1} due to the presence of hydroxyl group of an acid. Strong bands at 1730 cm^{-1} and 1704 cm^{-1} were indicative for the presence of carbonyl groups of ester and acid functional groups, respectively. A weak band at 1657 cm^{-1} was due to a double bond. The ^{1}H NMR spectrum (Appendix 4, Table 8) revealed the presence of eight singlets corresponding to methyl protons.

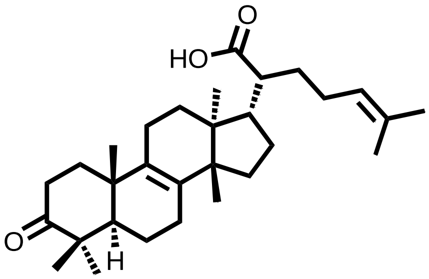
The singlet at δ 2.08 is due to an acetyl methyl group. The broad triplet at δ 4.67 indicated that the acetyl group is at the α orientation on C-3 [132]. The broad triplet appeared at δ 5.11(1H, *t, J* = 6.8 Hz, H-24) which was assigned to an olefinic proton. The triplet at δ 2.37 (1H, *t*, *J* = 7.6 Hz, H-20) is assigned to the methine proton on C-20 due to the electron withdrawing -COOH group.

The ^{13}C NMR spectrum of **169** revealed 32 carbon signals as presented in Table 8 (Appendix 4). DEPT-135 spectrum indicated the presence of eight methyl, ten methylene, five methine and nine quaternary carbon signals. The two most downfield signals which resonated at δ_C 170.9 and 182.4 were indicative of the presence acetyl and carboxylate carbons, respectively. Four carbon signals appeared in olefinic region suggesting the presence of two double bonds, but only one of these carbon signals could be attributed to a methine carbon based on the DEPT-135 spectrum. The carbon signal appeared at δ_C 123.5 is a characteristic signal of methine olefinic carbon in the side chain that attached to the tetracyclic ring system, whereas, the two typical signals resonated at δ_C 132.9 and 132.4 were suggestive for tirucallane type skeleton where the double bond in the ring system is between C-8 and C-9.

The deshielded carbon signal at δ_C 79.0 was assigned to C-3 where the acetyl group is attached. Base on the above spectroscopic data, **169** was identified as 3*α*-acetyl-tirucallic acid (3α-acetyl-tir-8,24-dien-21-oic acid). Comparison of the spectroscopic data with those reported for [31] 3*α*-acetyl tirucallic acid showed very close agreement (Table 8). **169** was first isolated from *B. serrata* [133].



169



166

Characterization of 3-oxo-tirucallic acid (166)

3-Oxo-tirucallic acid (**166**, BPK8) was isolated as a white powder. A strong deep-blue spot was observed on TLC (ss$_2$) at *Rf* = 0.35. The UV-Vis (EtOH) spectrum exhibited an absorption maximum at 248.1 nm. The IR spectrum displayed an absorption band at 3428 cm^{-1} due to the presence of hydroxyl group of the acid. A strong band at 1704 cm^{-1} was indicative of the presence of an acid carbonyl group. A medium absorption band at 1754 cm^{-1} was due to a ketone carbonyl group and a weak band at 1657 cm^{-1} was due to double bond.

Table 8: ^{1}H and ^{13}C NMR spectral data of **166** and **169**, and comparison with the data reported for 3-acetyl tirucallic acid and 3-oxotirucallic acid in the literature

400 MHz, CDCl$_3$, δ in ppm, *mult*., *J* in Hz

C	**169**		Literature		**166**		Literature	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1	1.25, 1.67	33.8	1.28, 1.74	34.9		35.6		35.7
2	1.60, 1.67	23.3	1.60, 1.68	23.4	2.48(*dd*)	34.6	2.5	34.6
3	4.67 (*t*)	79.0	4.50(*dd*)	80.5		218.0		216.9
4		37.1		37.8		47.3		47.3
5	1.23	49.6	1.22	51.0	1. 29	51.4	1.29	51.6
6	1.42, 1.67	18.6	1.42, 1.68	18.7		20.1		20.2
7	1.95, 2.07	27.0	1.93. 2.08	27.4		28.6		28.8
8		132.9		133.3		134.3		134.2
9		132.4		133.9		132.3		132.2
10		37.1		37.1		37.1		37.2
11	1.91, 2.01	21.4	1.91, 2.01	21.5		25.9		25.9
12	1.37, 1.67	29.3	1.37, 1.66	28.7		32.4		32.5
13		43.8		43.8		43.9		43.9
14		47.5		49.5		49.7		49.7
15	1.32, 1.57	29.7	1.30, 1.55	29.3		29.3		29.4
16	1.33, 0.95	25.9	1.34, 0.95	26.8		27.4	1.54	27.5
17	2.05	46.9	2.07	46.9	2.3	46.9	2.3	47.0
18	1.27(s)	19.9	0.94 (s)	20.0	0.83	21.1	0.83	21.2
19	0.88(s)	14.2	0.81 (s)	15.7	0.92	15.8	0.91	15.9

20	2.37	47.5	2.29	47.4	2.51(*m*)	47.5	2.5	47.6
21		182.4		182.0		182.3		182.4
22		32.4	1.56	32.4		21.3		21.4
23	1.95, 2.00	24.7	1.93, 2.00	25.9		26.9		26.9
24	5.11	123.5	5.09	123.5	5.11(*t*)	123.5	5.11	123.5
25		132.2		132.4		132.6		132.7
26	1.69(s)	25.7	1.68 (s)	25.6	1.69	25.7	1.68	25.7
27	1.60(s)	17.6	1.58 (s)	17.6	1.60	19.6	1.59	19.7
28	0.98(s)	15.9	0.87 (s)	16.6	1.06	24.2	1.05	24.3
29	0.92(s)	27.6	0.88 (s)	27.9	1.11	17.6	1.09	17.6
30	0.95(s)	24.4	0.88 (s)	24.4	1.05	26.5	1.04	26.2
31		170.9		170.0	-	-	-	-
32	2.08(s)	21.8	2.05(s)	21.3	-	-	-	-

The ^{1}H NMR spectrum (Appendix 5) of compound **166** showed seven singlets due to methyl protons. The desheilded methylene proton signal at δ 2.48 (2H, *dd*, *J* = 7.4, 4.0 Hz, H-2) is due to H-2. Three methine proton signals resonated at δ 1.29 (1H, *m*, H-5), δ 2.43 (1H, *m*, H-17) and δ 2.5 (1H, *m*, H-20) and were assigned for protons on C-5, C-17, and C-20, respectively. The triplet δ 5.11 (*t*, *J* = 7.2 Hz, H-24) was indicative of one olefinic proton.

The ^{13}C NMR spectrum revealed 30 carbon atom signals as presented in Table 8 and Appendix 5. DEPT-135 spectrum indicated the presence of seven methyl, ten methylene, four methine and nine quaternary carbon signals. The most downfield signal at δ_C 218.0 was a particular signal for ursane, oleanane and tirucallane type triterpenes with ketone group at C-3. The signal observed at δ 182.3 proved the presence of carbonyl carbon for a carboxylic acid.

Base on the spectroscopic data discussed above, **166** was identified as 3-oxo-tirucallic acid (3-oxo-tir-8,24-dien-21-oic acid). Comparison of the ^{1}H and ^{13}C data of **166** with those reported in the literature [31] for 3-oxo-tirucallic acid showed a very close resemblance (Table 8).

Inhibition of microsomal prostaglandin E_2 synthase-1 [134], an inhibitions of prostate cancer cell [103], anti-inflammatory activities of **166** and **169** have been reported [20].

Characterization of 3α-A-β-BA (122) and 3*α*-A-α-BA (147)

Compound **122** (BPK9) and compound **147** (BPK10) were isolated as an unseparable mixture. Analysis by TLC (ss_2) showed a deep purple spot at *Rf* = 0.25 and UV-Vis (EtOH) spectrum displayed an absorption peak at λ_{max} 249 nm.

The ^{1}H NMR spectrum (Appendix 6) showed a total of sixteen methyl proton resonances. Two proton signals at δ 5.09 (1H, *t*, *J* = 6.8 Hz, H-11 of A-β-BA) and 5.15 (1H, t, *J* = 3.2 Hz, H-11 of A-α-BA) were due to olefinic protons. The two proton signals at the most downfield region δ 5.31 (1H, *t*, *J* = 2.4 Hz, H-3 of A-β-BA) and 5.21 (1H, *t*, J = 2.8 Hz, H-3 of A-α-BA) were due to oxymethine protons. The ratio of compounds 122 1nd 147 in the mixture was estimated to be 1.0:0.67 (β:α) from the integration of the olefinic proton signals.

Eight methyl proton signals assigned for the β-isomer in the mixture were at δ 0.82 (*d*, *J* = 5.2 Hz, H-29), 0.83 (*s*, H-28), 0.92 (*d*, H-30), 0.94 (*s*, H-25), 1.05 (s, H-26), 1.13 (*s*, H-27), 1.25(s, H-23), 2.11 (*s*, H-32), whereas, the remaining methyl proton resonances assigned for the α-isomer were a δ 0.85 (s, H-28), 0.86 (s, H-28), 0.90 s, H-29), 0.97 (s, H-25), 1.01 (s, H-26), 1.15 (s, H-30), 1.20 (s, H-23), 2.10 (s, H-32). The presence of two intense doublets due to methyl protons was indicative of the fact that one of the compounds possessed a ursane-type skeleton.

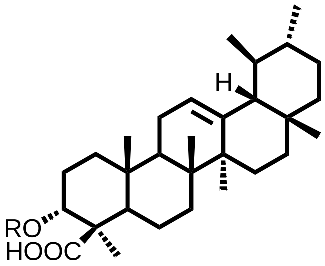
The ^{13}C NMR spectrum (Appendix 6) showed characteristic signals for two compounds. The two olefinic carbon signals at δ 124.5 and 139.5 are typical signals for β-boswellic acid (ursane-type skeleton) and also the two olefinic carbon signals at δ 121.8 and δ 145.0 are typical signals for α-boswellic acid (oleanane-type skeleton). Moreover, the presence of signals corresponding to two acetyl groups at δ 170.40 and 170.43, two carboxyl groups at δ 182.38 and 182.22 and two downfield oxygenated methine carbon signals at δ 73.04 and 73.23 were indicative for the presence of α- and β-boswellic acids.

The identity of compound **122** and **147** as 3α-acetyl-β-boswellic acid (3α-acetyl-urs-12-en-24-oic acid) and 3*α*-acetyl-α-boswellic acid (3α-acetyl-olean-12-en-24-oic acid), respectively, was further confirmed by comparing the ^{1}H and ^{13}C NMR data with those reported in the literature [74]. As shown in Table 9, the NMR data showed very close agreement. Both **122** and **147** were previously isolated from *B. serrata* [133].

These compounds were reported as inhibitors of TPA induced inflammation, COX-1 [65], human topoisomerases I and IIα [111] and also exhibited cytotoxic effects on human leukemia HL-60 cells [134, 135].

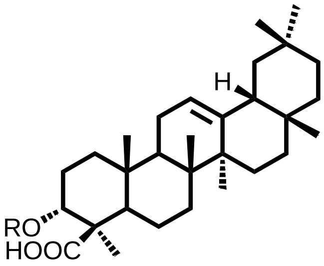
Characterization of β-Boswellic acid (121) and *α*-Boswellic acid (146)

Compound **121** (BPK12) and compound **146** (BPK13 were obtained as an inseparable mixture which showed a deep violet spot on TLC (ss$_2$, *Rf* = 0.30). The UV-Vis spectrum showed an absorption maximum 250 nm. The IR spectrum displayed an absorption band at 3428 cm^{-1} due to the hydroxyl group and carboxylic acid. The bands at 1704 cm^{-1} and 1626 cm^{-1} were due to a carbonyl group and a double bond.



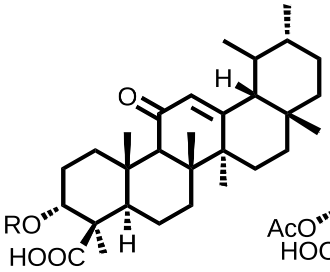
R = H, **121**

R = OAc, **122**



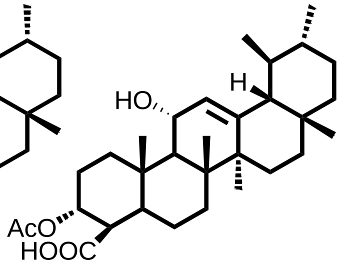
R = H, **146**

R = OAc, **147**



R = H, **123**

R= OAc, **124**



132

The ^{1}H NMR spectrum (Appendix 8) revealed that compound **121** (β-BA) was the as major component of the mixture and showed seven intense methyl proton signals appeared at δ, 0.89 (3H, *s*, H-28), 1.02 (3H, *s*, H-25), 1.06 (3H, *s*, H-26), 1.11 (3H, *s*, H-27), 1.37 (3H, *s*, H-23), 0.82 (3H, *d*, H-29) and 0.92 (3H, *d*, H-30). The broad singlets at δ 4.10 and δ 4.14 could be attributed to the protons attached to the oxygenated carbons (C-3) of **121** (β-BA) and **146** (α-BA), respectively. The olefinic proton signal of **121** was observed at δ 5.16 (1H, *t, J* = 3.6 Hz, H-12), whereas, the olefinic proton of **146** resonated at δ 5.2 (1H, *t, J* = 3.6 Hz, H-12). The OH proton signal of the mixture appeared at δ 3.51(*s*). The ratio of **121**:**146** was determined to be 2:1 from the integration of the olefinic proton (H-12) signal in the 1H NMR spectrum of the mixture.

The ^{13}C NMR spectrum revealed 30 major carbon signals as presented in Appendix 8. The DEPT-135 spectrum indicated the presence of seven methyl, nine methylene, seven methine and seven quaternary carbon signals. The characteristic signals in the olefinic region were indicative of ursane- (δ 124.50 and δ 139.61) and oleanane- (δ 121.7 and 145.1) type skeleton. The remaining minor signals are suggestive of the presence of **146** in the mixture. Moreover, the oxygenated carbon signals at δ 70.7 and 70.8 corresponds to the oxymethine carbons (C-3) and the signals at δ 182.93 and 182.96 are due to the carboxyl group in **121** and **146**, respectively.

The NMR spectral data of the mixture of **121** and **146** resembled the NMR data generated for the mixture of **122** and **147** except for the absence of signals for acetyl groups. Hence **121** and **147** are deacetylated forms of **122** and **147**, respectively. Table 9 [136] shows a comparison of the ^{13}C NMR data of **121** and **146** with the data reported for β-boswellic acid and α-boswellic acid, respectively, and a very close agreement is observed. Hence **121** was identified as β-boswellic acid and **146** was identified as α-boswellic acid.

Anti-inflammatory [86], anticancer [66], antitumor [134, 137] and alleviative activities of these two compounds were reported.

Characterization of 3*α*-acetyl-11-keto-β-boswellic acid (124)

AKBA (**124,** BPK11) was obtained as white fine crystal with a melting point of 261-265 ^{0}C (*Lit.,* [21] 271-274 ^{0}C) and strong UV absorption maximum at 250 nm in EtOH. Analysis by TLC (ss$_2$) showed a strong dark spot at *Rf* = 0.36 (UV at λ_{max} 254 nm). It exhibited an IR absorption band at 3434 cm^{-1} due to hydroxyl group of carboxylic acid. The band at 1724 cm^{-1} revealed the presence of an α,β-unsaturated carbonyl group and the strong bands at 1733 cm^{-1} and 1705 cm^{-1} confirmed the presence of ester and acid functional groups, respectively. The weak band at 1644 cm^{-1} is indicative of the presence of a double bond.

^{1}H NMR spectrum (Appendix 7) of compound **124** showed eight methyl proton resonances. The presence of three-proton doublets at δ 0.82 (*J* = 6.4 Hz, H-29) and 0.96 (J = 2 Hz, H-30) were suggestive of a ursane-type skeleton. The methylene proton signals at δ 2.10 (*dt*, *J* = 8.0, 4.0 Hz), 2.26 (*td*, *J* = 3.2, 14.8 Hz) and 2.55 (*dt*, *J* = 13.2, 3.3 Hz) were assigned to H-16, H-2 and H-1, respectively. The one-proton singlet at δ 2.43 (1H, *s*, H-9) is a characteristic signal of 11-keto functionalized system of ursane-type triterpenes. The downfield broad triplet at δ 5.32 (*J* = 2.2 Hz, H-3) was indicative of the presence of a β oriented oxymethine proton on C-3. The most downfield singlet at δ 5.58 (H-12) was due to an olefinic proton on C-12, thus suggesting the absence of proton on the adjacent carbons C-11 and C-13. Thus, the carbonyl group was placed at C-11.

The ^{13}C NMR spectrum revealed 32 carbon signals as presented in Appendix 7. The DEPT-135 spectrum indicated eight methyls, eight methylene, seven methine and nine quaternary carbons. The signals resonated at δ 170.2, 181.5 and 199.2 were assigned to acetyl, carboxylic and ketone carbonyl carbons. The olefinic carbon signals appeared at δ_C 130.5 and 165.0 were characteristic signals of ursane-type triterpene with a ketone function at C-11.

The position of the acetyl group at C-3 was determined through an HMBC experiment in which the oxymethine proton signal at δ 5.32 (1H, *bt*, H-3) showed 2_{JC}-H correlations with C-2 (δ_C 23.5) and C-4 (δ_C 23.8), and 3_{JC}-H correlations with C-1 (δ_C 33.9), C-5 (δ_C 50.4) and C-23 (δ_C 23.8); while the oxygenated methine carbon signal at δ_C 73.0 (C-3) showed 3_{JC}-H interactions with H-1 (δ 2.55), H-5 (δ 1.36) and H-23 (δ 1.21).

Table 9: ^{13}C NMR spectral data of **121**, **122**, **146** and **147**, and comparison with the data reported for β-ABA, α-ABA, α-BA and β-BA in the literature

400 MHz, CDCl$_3$

C	**122**	β-ABA [74]	**147**	α-ABA [74]	**146**	α-BA [136]	**121**	β-BA [136]
1	34.5	34.5	34.0	34.3	33.6	33.6	33.9	33.9
2	23.7	23.6	23.5	23.6	26.2	26.2	26.2	26.2
3	73.0	73.4	73.2	73.6	70.7	70.8	70.7	70.8
4	46.6	46.7	46.8	46.7	47.4	47.4	47.4	47.4
5	50.6	50.6	50.4	50.6	49.1	49.1	49.1	49.1
6	19.6	19.6	16.8	16.7	19.7	19.7	19.7	19.7
7	33.1	33.1	32.8	32.8	33.1	32.7	33.3	33.1
8	40.0	40.0	39.8	39..8	39.7	39.8	40.0	40.0
9	46.9	46.9	46.8	46.8	46.7	46.7	46.8	46.8
10	37.4	37.4	37.6	37.5	37.6	37.6	37.5	37.5
11	23.3	23.4	23.4	23.5	23.5	23.5	23.27	23.4
12	124.5	124.5	121.8	121.8	121.7	121.7	124.5	124.5
13	139.5	139.5	145.0	145.0	145.1	145.1	139.6	139.6
14	42.3	42.3	43.9	44.9	41.9	41.9	42.3	42.3
15	26.5	26.5	25.9	26.1	26.2	26.0	26.5	26.5
16	28.1	28.1	27.1	27.0	26.9	26.9	28.1	28.1
17	33.8	33.8	32.5	32.5	32.5	32.5	33.8	33.8
18	59.2	59.2	47.4	47.4	47.4	47.3	59.2	59.2
19	39.6	39.6	47.3	46.9	46.7	46.7	39.7	39.7
20	39.7	39.7	31.1	31.0	31.3	31.1	39.6	39.6
21	31.2	31.2	34.7	34.7	34.7	34.7	31.3	31.3
22	41.5	41.5	37.2	37.1	37.1	37.1	41.5	41.5
23	23.6	23.6	23.3	23.7	24.1	24.2	24.1	24.2
24	182.3	182.3	182.2	182.2	182.9	183.2	182.9	183.1
25	13.2	13.3	13.0	13.2	13.3	13.1	31.1	13.3
26	17.5	16.9	16.9	16.5	16.7	16.7	16.9	16.9
27	22.2	23.2	25.7	25.9	25.9	25.9	23.2	23.2

28	28.8	28.7	28.4	28.4	28.4	28.4	28.8	28.8
29	17.7	17.4	24.4	23.6	33.3	33.3	17.4	17.4
30	21.4	21.3	33.3	33.3	23.7	23.7	21.4	21.4
31	170.4	170.3	170.4	170.3	-	-	-	-
32	21.4	21.3	21.1	21.3	-	-	-	-

The configuration of the acetyl group at C-3 was assigned to be α-oriented on the basis of the absence of interactions between H-3 and H-5 in the COSY experiment [135]. Compound **124** was first isolated from *B. serrata* in 1978 and was reported as 3*α*-acetyl-11-keto-β-boswellic acid (3α-acetyl-11-oxo-urs-12-en-24-oic acid) [133]. The ^{1}H and ^{13}C NMR data of **124** were the data reported for 3*α*-acetyl-11-keto-β-boswellic acid and in the literature [74, 138] and a very close resemblance was observed as shown in Table 10.

The biological activity of **124** was extensively studied [84]. This included studies of its anti-inflammatory [20, 112, 123, 134, 139], anti-tumor [85], anti-cancer [66, 67], anti-ulcer [96], anti-staphylococcal [122] and anti-microbial properties [123]. Its ability to inhibit leukotriene biosynthesis [105] was also investigated. 124 was also described as an inhibitor of topoisomerase I and IIα [84, 140].

Characterization of 11-keto-β-boswellic acid (123)

11-Keto-β-boswellic acid (**123**, BPK15) was isolated as white powder with UV absorption maximum at 249.5 nm in EtOH. The strong dark spot under UV light at 254 nm with at *Rf* = 0.25 on TLC (ss_2) without spraying agent is a characteristic spot for this compound. It exhibited a characteristic absorption band at 3454 cm^{-1} in it's the IR spectrum indicating the presence of O-H. The absorption band at 1752 cm^{-1} was due to α,β-unsaturated C=O group and the band at 1708 cm^{-1} indicated presence of COOH group. Furthermore, the conjugated C=C vibration was detected at 1669 cm^{-1} as a weakly intense band.

The ^{1}H NMR spectrum (Appendix 10) of **123** showed seven methyl proton resonances of which two appeared as doublets were suggesting a ursane skeleton. The broad triplet at δ 4.1 was assigned to H-3. The ^{1}H NMR data of **123** resembled that of **124** except for the absence of signals for acetyl group in the spectrum of **123**. The ^{13}C NMR spectrum revealed 30 carbon signals as presented in Table 10 and Appendix 10. The DEPT-135 spectrum of **123** indicated the presence seven primary, eight secondary, seven tertiary and eight quaternary carbon atoms in the compound. The ^{13}C NMR data of **123** was comparable with that of **124** except for the luck of signals for acetyl group. Based on the spectroscopic data, compound 123 was identified as 11-keto-β-boswellic acid.

Table 10: ^{1}H and ^{13}C NMR spectral data of **123**,**124** and **132**, and comparison with the data reported for AKBA, KBA and 11-hydroxy-ABA in the literature

400 MHz, $CDCl_3$, δ in ppm, *mult*., *J* in Hz

CN	**124**		AKBA		**123**		KBA		**132**	11-OHABA
1	2.55(*dt*)	34.6	2.53	34.6	2.52 (*dt*)	33.8	1.32-2.51	33.9	33.7	39.7
2	2.26(*td*)	23.5	2.22	23.5	2.28-2.37	26.2	1.57-2.30	26.3	26.6	27.5
3	5.32	73.0	5.28	73.2	4.10 (*bt*)	70.5	4.07	70.5	73.2	78.8
4		46.4		46.5		47.2		47.3	46.7	39.1
5	1.36	50.4	1.37	50.5	1.42-1.49	48.8	1.41-1.49	48.9	50.6	55.6
6	1.85	18.7	1.86	18.8	1.85-1.72	18.8	1.84-1.71	18.9	19.4	18.5
7	1.65	32.8	1.65	32.8	1.65	32.9	1.65	32.9	36.4	33.5
8		45.1		45.1		45.1		45.1	43.2	43.3
9	2.43(*s*)	60.3	2.39	60.3	2.44 (*s*)	60.4	2.42	60.5	54.4	55.8
10		37.4		37.4		37.5		37.6	38.5	38.1
11		199.2		199.2		199.5		199.4	68.6	68.3
12	5.58(*s*)	130.5	5.53	130.5	5.56 (*s*)	130.5	5.54	130.6	128.8	128.6
13		165.0		164.8		165.1		164.9	142.8	143.1
14		43.7		43.7		43.8		43.8	42.3	42.1
15	1.89	27.2	1.89	27.2	1.27-1.45	27.2	1.22-1.92	27.2	27.9	27.9
16	2.19	27.5	2.16	27.5	1.00- 2.08	27.5	1.00 -2.08	27.6	27.9	27.4
17		34.0		33.9		33.9		34.0	36.4	33.6

18	1.50	59.0	1.52	59.0	1.51 (*d*)	59.0	1.51	59.1	58.2	58.0
19	1.41	39.3	1.40	39.3	1.39 (*bt*)	39.2	1.37-1.39	39.3	39.4	39.3
20	0.91	39.3	0.93	39.3	0.96 (*m*)	39.2	0.93	39.3	39.4	39.4
21	1.41	30.9	1.44	30.9	1.32 -1.44	30.9	1.32-1.49	30.9	31.1	31.1
22	1.44	40.9	1.47	40.9	1.41	40.9	1.35-1.41	40.9	41.3	41.3
23	1.2(*s*)	23.8	1.21	23.8	1.37	24.3	1.33	24.3	24.0	28.7
24		181.5		181.7		182.1		182.7	181.3	
25	1.16(*s*)	13.2	1.12	13.2	1.15	13.2	1.12	13.2	14.2	16.8
26	1.26(*s*)	18.3	1.17	18.4	1.20	18.3	1.17	18.4	18.0	18.0
27	1.36(*s*)	20.5	1.32	20.5	1.33	20.5	1.30	20.6	21.3	23.1
28	0.84(*s*)	28.8	0.81	28.8	0.84	28.8	0.81	28.9	28.7	28.2
29	0.80(*d*)	17.4	0.79	17.4	0.82	17.4	0.78	17.4	17.5	17.6
30	0.96(*d*)	21.1	0.93	21.1	0.96	21.1	0.93	21.1	21.4	21.4
31		170.2		170.2					170.3	
32	2.1(*s*)	21.3	2.06	21.3					23.0	

The spectroscopic data generated for **123** was compared with the data reported for 11-keto-β-boswellic acid (3-α-hydroxy-11-oxo-urs-12-en-24-oic acid) and a very close agreement was observed as shown in Table 10 [138]. **123** was first isolated and reported by Pardhy in 1978 from *B. serrata* [133] and later by Paul in 2012 from *B. sacra,* and *B. papyrifera* [24]. Anti-inflammatory, anti-tumor, anti-cancer and anti-ulcer activities of **123** were extensively studied. **123** found to have less potency than AKBA and higher efficacy than other derivatives of boswellic acids [84].

Characterization of 3*α*-acetyl-11-hydroxy-β-boswellic acid (132)

3*α*-Acetyl-11-hydroxy-β-boswellic acid (**132**, BPK14) was obtained as a white powder after purifying by PTLC. It had a with melting point 192-195 ^{o}C (*Lit.* [21], 195-197 ^{o}C) and UV absorption maximum at 248.5 nm in EtOH. It gave a strong violet spot on TLC (ss$_2$) at *Rf* = 0.25 with vanillin/H_2SO_4 reagent. The IR spectrum revealed the presence of hydroxyl group due to characteristic medium broad absorption band at 3442 cm^{-1}. The presence of an ester function and a double bond were confirmed by the bands at 1708 cm^{-1} and at 1626 cm^{-1}, respectively.

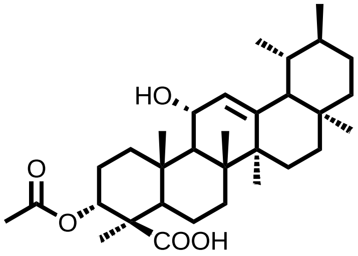
The ^{1}H NMR spectrum (Appendix 9) of **132** showed eight methyl proton signals of which two appeared as doublets. An olefinic proton signal was observed at δ 5.20 (1H, *d*, J = 2.8 Hz, H-12). The splitting pattern, as well as the desheilded chemical shift of this signal indicated the presence of an electron withdrawing substituent at C-11. Two oxymethine proton signals were observed at δ 5.31 (*bt*, H-3) and 4.28 (*dd*, *J* = 9.0, 2.8 Hz, H-11).

The oxymethine proton resonance at δ 4.28 was assigned to proton at C-11 which coupled with H-12 (*J* = 9.0 HZ) and H-9 (*J* = 2.8 HZ). The one-proton doublet at δ 2.15 (*J* = 2.8 HZ) was attributed to H-9. The singlet δ 2.18 was due to the C-11 hydroxyl proton. The signals at δ 2.09 (*m*), 2.01(*dd, J* = 11.4 and 4.0 Hz, H-19) and 1.85 (*m*) were methine protons of H-9, H-18 and H-20, respectively.

The ^{13}C- NMR spectrum of **132** revealed 32 carbon signals as presented in Appendix 9. The DEPT-135 spectrum indicated the presence of eight methyl, eight methylene, eight methine and eight quaternary carbons in the molecule. Two signals at δ 68.6 (C-11) and 73.2 (C-3) were indicative of the presence of two oxygenated methine carbons, which agreed with the observation of two oxygenated methine proton signals at δ 5.31 and 4.28, respectively, in the ^{1}H NMR spectrum.

Two olefinic carbon resonances appeared at $\delta_{C\text{-}12}$ 128.8 and $\delta_{C\text{-}13}$ 142.8 indicating the presence of only one double bond. The most downfield carbon signals at δ_{C} 170.3 and 181.3 were assigned to the acetoxy and carboxylic acid groups in the molecule, respectively.

Based on the information extracted from NMR data the compound would be an ursane-type triterpene acid with a hydroxyl group at C-11. Thus, the compound was proposed to be 3*α*-acetyl-11-hydroxy-β-boswellic acid (3α-acetyl-11-hydroxy-urs-12-en-24-oic acid).



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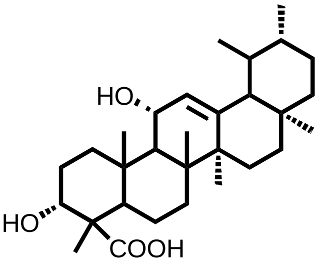

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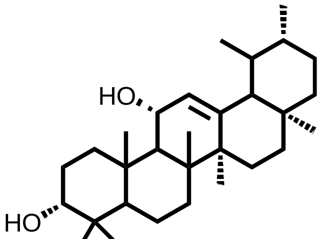

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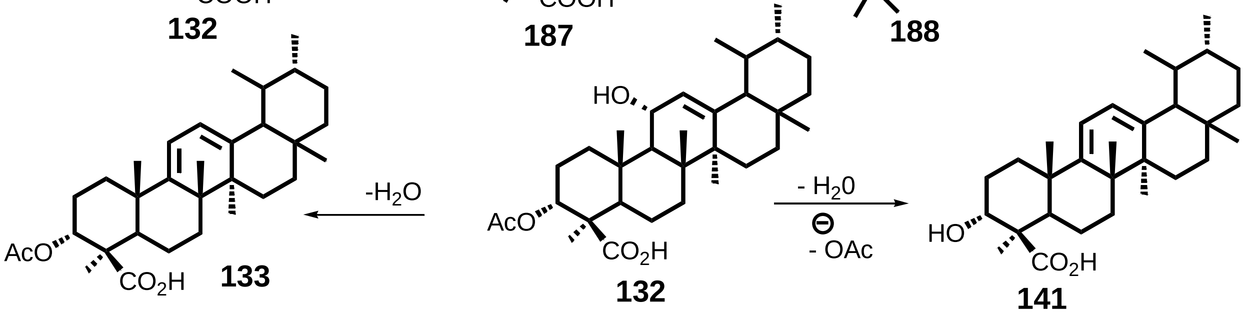


133 **132** **141**

Scheme 4: Biosynthesis of compound **133** and **141** from **132**

Table 10 gives a comparison of the NMR data of 132 with those of 187 and 188 [141, 142]. Compound **132** was reported from the resin of *B. serrata* and was presumed to be the precursor of 3*α*-acetyl-9,11-dehydro-β-boswellic acid (**133**) and 9,11-dehydro-β-boswellic acid (**141**). Degradation of the natural compound **132** via dehydration resulted in the thermodynamically more stable products **133** and **141** as shown in *Scheme 4* [65].

To summarize this section, a total of twelve compounds were isolated from the resin of *B. papyrifera* Kebtele. One fatty acid and nine triterpenic acids were isolated for the first time from this resin, of which two tirucallic acid and seven boswellic acid derivatives were reported to be active ingredients for the treatment of inflammation and cancer [84, 103]. In this work, only the major components were isolated from 4 g of both the neutral and acid fractions.

The TLC of the EtOH extract of the bark was compared with the EtOH extract of the resin and the major compounds were found to be identical.

2.2.2. Compounds isolated from *B. papyrifera* resin

The resin of B. papyrifera was extracted with EtOH and the crude extract was subjected to phytochemical studies. The TLC of the crude extract (*Fig. 7*, 13a and 13b) showed predominantly two spots. From the neutral fraction of this resin (BpRn), verticillan-4,7(20),11-triene (**104**, BP1), incensole acetate (**111**, BP2) and incensole (**109**, BP3) were isolated, and characterized as described below.

The acid fraction of the resin (BpRa) was rich in tetra and pentacyclic triterpenes. Repeated purification by column chromatography furnished **169** (BP4), **166** (BP5), a mixture of **122** (BP6) and **147** (BP7), **124** (BP8), a mixture of **121** (BP9) and **146** (BP10), and **123** (BP11). The compounds isolated from the acid fraction of this resin and the resin of *B. papyrifera* Kebtele resin were almost the same. However, the neutral fractions of these resins are different. The TLC of the EtOH extract of the bark of *B. papyrifera* showed spots with the same Rf value as verticillan-4,7(20),11-triene (**104**), incensole acetate (111) and some boswellic acids [143].

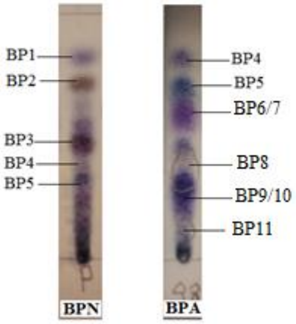


Figure 7: TLC chromatogram of EtOH extract of *B. papyrifera* resin: (ss_1, ss_2)

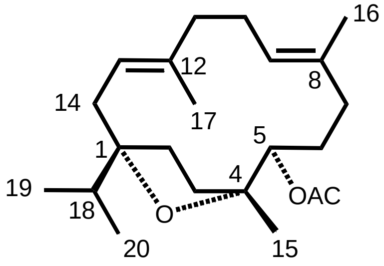
The fatty acid eluted together with **166** and **169** was converted in to ester **190** by reflexing the mixture in MeOH and H_2SO_4 for 2 h. The resulting mixture was subjected to silica gel column chromatography and **190** was obtained as a yellowish oil.

Characterization of Incensole acetate (111)

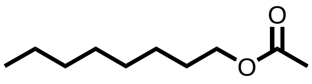
Incensole acetate (**111**, BP2) was isolated as colorless viscous oil. It exhibited an IR absorption band at 1743 cm^{-1} due to the presence of an ester carbonyl group. The weak band at 1657 cm^{-1} indicated the presence of a C=C bond stretching. The ^{1}H NMR spectrum (Appendix 11) showed a total of six methyl group resonances which appeared at δ 0.89 (3H, *d*, *J* = 4 Hz, H-19), 0.91 (3H, *d*, *J* = 3.6 Hz, H-20), 1.10 (3H, *s*, H-15), 1.54 (3H, *s*, H-17), 1.58 (3H, *s*, H-16) and 2.06 (3H, *s*, H-22). The signals due to two olefinic protons were overlapped and appeared as a broad singlet at δ 5.16 (2H, H-9, and H-13). The oxymethine proton resonance was observed at δ 4.88 (1H, *d*, *J* = 10.4 Hz, H-5).

Twenty two carbon atom signals were found in the ^{13}C NMR spectrum as presented in Appendix 11. The DEPT-135 spectrum of **111** indicated the presence of six methyl, seven methylene, four methine and five quaternary carbons in the molecule. The most down field signal at δ_C 171.1 was assigned an ester carbonyl group. Four olefinic carbon signals appeared in the olefinic region which proved the presence of two double bonds in the molecule. The aliphatic carbon signals at δ 76.4, 83.0, and 89.2 were attributed to the oxygenated carbon atoms C-5, C-4 and C-1, respectively.

Based on the above spectroscopic data, compound **111** was identified as incensole acetate. The isolation and structural elucidation of **111** was first described by Nicoletti in 1967 [144]. The NMR data was compared with the data reported by Paul [24] in the literature and very close agreement was found as shown in Table 11.



R = OAc, **111**
R = H, **109**



189

Octyl acetate (**189**) was eluted together with incensole acetate (**111**) in small quantities. Purification by silica gel column chromatography gave 198 as oil.

Characterization of Incensole (109)

Compound **109** (BP3) was isolated as a yellowish viscous oil. The IR spectrum showed an absorption band at 3409 cm^{-1} due to O-H stretching. The strong absorption band at

1695 cm^{-1} was indicative of C=C bond stretching. The ^{1}H NMR spectrum (Appendix 12) revealed five methyl proton signals. The proton signal at δ 3.28 (1H, d, *J* = 10 Hz, H-5) was assigned to the oxymethine proton on C-5. The signals due to the olefinic protons appeared as overlapping multiplet at δ 5.05.

The ^{13}C NMR and DEPT-135 spectra revealed the presence of five methyl, seven methylene, four methine and four quaternary carbons as shown in Table 11 and Appendix 12. The signals at δ 121.2, 125.1, 134.1 and 134.2 were indicative of the presence of two double bonds in the molecule. Three carbon signals appeared at δ 75.4, 84.3 and 88.3 which were assigned to the three oxygenated carbons C-5, C-4 and C-1, respectively. Comparison of the 1H and 13C NMR data of 109The data is compa with those reported for incensole by Paul [24] (Table 11) showed a very close agreement. Hence, **109** was identified as incensole.

The anti-inflammatory potentials of incensole acetate and incensole were published in 2006 [14, 87]. It is believed that incensole enhances the euphoric feeling produced during religious functions [2, 145]. The neuroprotective property of incensole against traumatic brain injury (TBI) was studied in 2008 [146]. The anti-depressant property of incensole acetate was reported in 2012 [147]. It was also shown to inhibit neoplastic cells, prevent precancerous lesions, tumors, cancer growth [148].

Table 11: ^{1}H and ^{13}C NMR assignments for **108**, **109** and **111**, and comparison with the data reported for serratol, incensole and incensole acetate in the literature

400 MHz, CDCl$_3$, δ in ppm, *mult*., *J* in Hz

CN	**111**		In-OAc [24]		**109**		incensole [24]		**108**		serratol [24]	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1		89.2		89.2		88.3		88.5		76.8		76.8
2	1.75,1.62	30.2	1.78, 1.61	30.3	1.82, 1.59	30.5	1.84, 1.59	30.6	2.14, 2.20	34.7	2.14, 2.20	34.7
3	1.89,1.57	35.5	1.89, 1.59	35.5	2.05, 1.74	36.3	2.06, 1.74	36.3	5.25 (*t*)	120.9	5.24	120.8
4		83.0		83.0		84.1		84.1		136.5		136.5
5	4.88(*d*)	76.4	4.87	76.5	3.28	75.4	3.30	75.5	2.21	39.4	2.21	39.4
6	1.85,1.51	27.6	1.85,	27.7	1.88,	30.6	1.89,	30.7	2.30	24.8	2.30	24.7

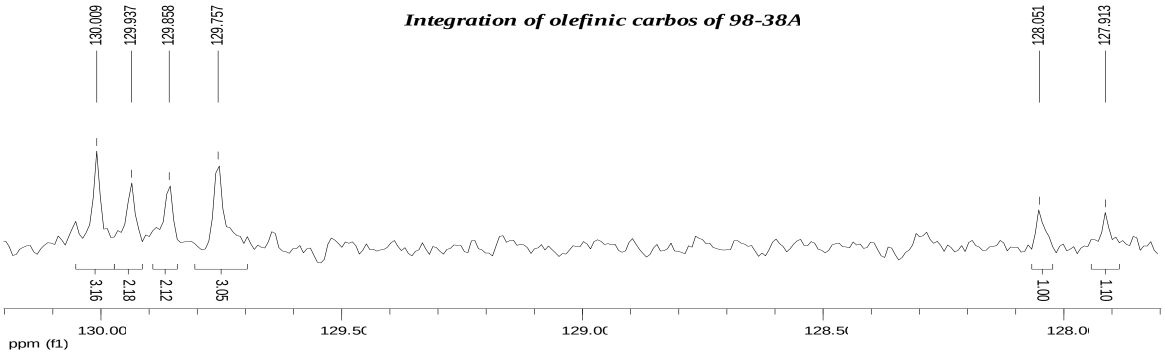
			1.51		1.32		1.32					
7	2.05, 1.64	33.3	2.02, 1.63	33.3	2.14, 1.99	33.6	2.12, 1.99	33.6	4.90(*t*)	125.9	4.88	125.9
8		133.1		133.2		134.1		134.1		133.2		133.2
9	5.16(*bs*)	125.3	5.18	125.3	5.05	125.1	5.08	125.1	1.95, 2.11	39.9	1.95, 2.11	39.8
10	2.19	24.7	2.19	24.7	2.10- 2.19	24.8	2.11- 2.18	24.8	2.09	23.7	2.09	23.7
11	2.16	38.4	2.15	38.4	2.10- 2.16	38.5	2.11- 2.16	38.6	5.00(*t*)	123.6	4.99	123.1
12		135.2		135.2		134.2		134.2		135.5		135.5
13	5.16(bs)	120.9	5.16	121.0	5.11	121.1	5.12	121.7	1.75, 1.93	33.4	1.75, 1.93	33.4
14	2.17	31.9	2.17	32.0	2.03, 2.19	32.7	2.04, 2.17	32.3	1.65	34.9	1.65	34.9
15	1.10	22.0	1.11	22.0	1.06	20.6	1.07	20.6	1.71	34.5	1.71	34.5
16	1.58	17.6	1.59	17.6	1.62	18.1	1.62	18.1	1.55	15.0	1.54	15.0
17	1.54	16.0	1.55	16.0	1.49	16.5	1.5	16.1	1.57	15.1	1.56	15.1
18	1.89	34.8	1.9	34.9	1.91	34.7	1.90	34.8	1.59	16.3	1.58	16.3
19	0.89(*d*)	17.9	0.89	18.0	0.89	17.7	0.89	17.9	0.96(*d*)	16.6	0.94	16.6
20	0.91(*d*)	18.0	0.91	18.0	0.90	18.0	0.91	18.0	0.92(*d*)	16.8	0.92	16.7
21		171.1		171.1	-	-	-	-	-	-	-	-
22	2.06	21.2	2.05	21.2	-	-	-	-	-	-	-	-

Characterization of docosahexaenoic acid ester (190)

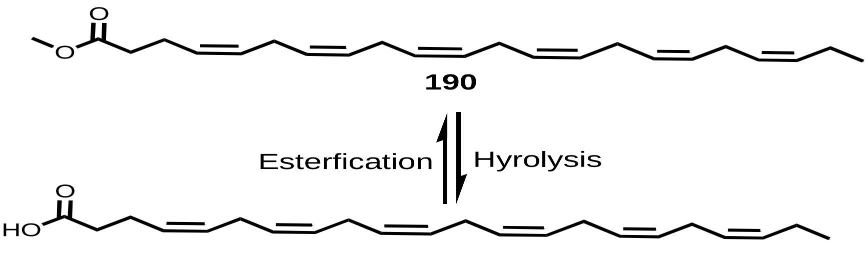
The EIMS of compound **190** indicated molecular ion peak at *m/z* 342 corresponding to the molecular formula $C_{23}H_{34}O_2$. ^{1}H NMR spectrum (Appendix 19) of compound **190** showed a terminal methyl proton signal at δ 0.89 as a triplet. The two proton triplet at δ 2.31 (2H, *t, J* = 7.6 Hz, H-2) was due to the methylene protons attached to the α-C of the carboxylic group. The broad triplet at δ 5.36 was indicative of the presence of olefinic protons. The most intense proton signal at δ 3.68 (3H, *s*, OCH_3) was due to the methoxy group that confirmed the presence of a methyl ester. The doublet of doublet δ

2.00-2.05 (J = 6.0 and 5.6 Hz) was indicative of the two methylene protons found in between the double-bonded carbons. The signal at δ 1.63 appeared as a doublet of triplets and was assigned to the remaining methylene protons adjacent to the double bonded carbons. The remaining proton signal appeared as a broad singlet at δ 1.26.

The ^{13}C NMR spectrum of **190** revealed 17 carbon signals as presented in Appendix 19. The DEPT-135 spectrum indicated two methyl carbon signals at δ 14.13 and 51.44 corresponding to the terminal methyl and methoxy carbons, respectively. Six overlapping carbon signals were observed in the olefinic region (δ 127.91-130.00) with different intensities. The carbon signals resonances at δ 127.91 and 128.08 were due to one carbon each while the signals at δ 129.85 and 129.93 were due to two carbons each. The carbon signals at δ 129.75 and 130.00 were due to three carbons each, accounting for a total of twelve double-bonded carbons. The signal which appeared at δ 174.36 was suggestive of an ester functional group.



The molecular formula $C_{23}H_{34}O_2$ can be assigned to **190** based on EIMS and NMR information. Hence, **190** was characterized as 4, 7, 10, 13, 16, 19-docosahexaenoic acid methyl ester (22:6).



Docosahexaenoic acid

As summary for this section; the chemical profiles of *B. papyrifera* Kebtele resin and *B. papyrifera* resin are different due to the presence of cneorben-10-ol (**119**) in Kebtele resin, and incensole (**104**) and incensole acetate (**111**) in the resin of *B. papyrifera*. Tetra- and pentacyclic triterpenic acids isolated from the two resins were found to be the same. As claimed by Asmare [10] the *Boswellia* species from Kebtele may be either a different species or a subspecies of *B. papyrifera*, commonly known as Tigray-type. All tetra- and pentacyclic triterpenic acids isolated from this resin were reported from the same resin by Paul in 2012 [24, 48, 149].

2.2.3. Compounds isolated from *B. sacra (syn. B. carteri)* resin

From the neutral fraction *B. sacra* resin (BsaRn), a marker compound, serratol (**108**, Bsa3) was isolated which was observed as a yellow spot on TCL as shown in *Fig. 8*. The presence of tirucallic acids (**166** and **169**) and boswellic acids (**122**,**147**, **124**, **121, 146**, and **123**) in the acid fraction (BsaRa) was confirmed by ^{1}H and ^{13}C NMR data analysis together with TLC.

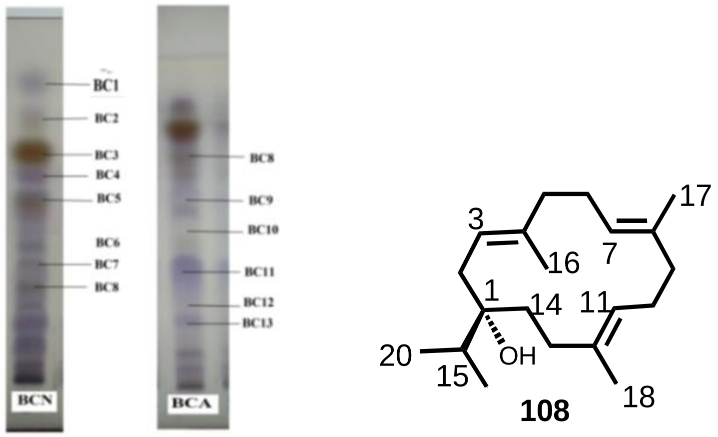


Figure 8: TLC (Left: ss$_1$; Right: ss$_2$) chromatogram of EtOH extract of B. sacra

Characterization of serratol (108)

Serratol (**108**, BSa3) was obtained as yellowish viscous oil. It exhibited IR absorption bands at 3473 cm^{-1} and1657cm^{-1} that suggested the presence of O-H and C=C bond.

The ^{1}H NMR spectrum (Appendix 13) of **108** showed a total of five methyl proton resonances at δ 0.93 (3H, *d*, *J* = 7.2 Hz, H-20), 0.96 (3H, *d*, *J* = 6.4 Hz, H-19),1.55 (3H, *s*, H-16) 1.57 (3H, *s*, H-17) and 1.59 (3H, *s*, H-18). Three olefinic methine proton signals were observed at δ 5.25 (1H, *t*, *J* = 7.6 Hz, H-3), 5.00 (1H, *t*, *J* = 6.8 Hz, H-11) and 4.90 (1H, *t*, *J* = 6.4 Hz, H-7).

The ^{13}C NMR spectrum revealed 20 carbon signals as presented in Appendix 13. The DEPT-135 spectrum indicated the presence of five methyl, seven methylene, four methine and four quaternary carbon atoms in the molecule. Six carbon resonances were observed in the olefinic region suggesting that the presence of three double bonds. The oxygenated carbon signal at δ 76.8 was due to C-1. Thus, based on the above spectroscopic data of **108** was identified as serratol.

The first isolation and structural elucidation of serratol (S-(-)-cembra-3E,7E,11E-triene-1-ol) was reported by Pardhy *et al.* in 1978 from *B. serrata* [150]. Paul also isolated serratol from *B. sacra (*Syn: *B. carteri)* in 2012 [24]. The complete structural assignment of serratol was reported by Schmidt *et al.* in 2011 [104].

The chemical shifts of the carbons and protons of **108** were compared with the data reported for serratol [24] as shown in Table 11 and complete agreement was observed. serratol was reported to have neuroactive, anti-inflammatory [151] and anti-plasmodial [104] activities.

2.2.4. Compounds isolated from *B. frereana* resin

The TLC (ss$_1$, *Fig. 9, 13a* and *13b*) of the crude EtOH extract showed the presence of four strong spots after spraying with vanillin/H_2SO_4 reagent. From the neutral fraction of the resin (BfRn), α-phellandrene dimer (**118**), epilupeol acetate (**163**), epilupeol (**158**) and lupeol (**159**), were isolated. The major compound in the resin was epilupeol (**158**) [100]. Following the extraction method discussed in Section 4.2.3, the amount of acid fraction obtained was very small. Hence, the chemical investigation on the acid fraction was not performed. The *α*-amyrin triterpenes possess the ursane- type basic skeleton and the *β*-amyrin triterpenes possess of the oleanane-type basic skeleton, and the only difference between them is the methyl position in the E-ring. However, the situation is vice-versa β- and α-boswellic acids, respectively.

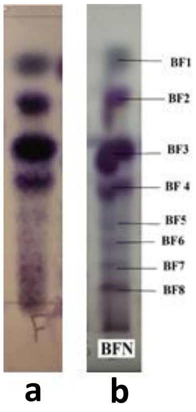


Figure 9: TLC (ss_1) chromatogram of the EtOH extract of *B. frereana*

*(*a: EtOH extract, b: neutral fraction*)*

Characterization of α-Phellandrene dimer (118)

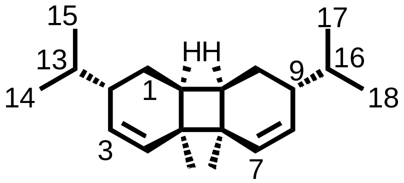
α-Phellandrene dimer (**118**, BF1), was isolated as yellowish viscous oil. A light blue spot was observed on TLC at *Rf* = 0.71. It exhibited an IR absorption band at 1665 cm^{-1} which confirms the presence of double bond. The ^{1}H NMR spectrum (Appendix 14) of **118** showed a total of three methyl proton signal at δ 0.90 (*d*, H-15/17), 0.92 (*d*, H-14/18) and 0.94 (*s*, H-19/20). Two olefinic proton signals were observed at δ 5.71 (1H, *d*, *J* = 10.4 Hz H-4) and δ 5.58 (1H, *dd*, *J* = 2.8, 7.6 Hz, H-3). The aliphatic methine proton signals appeared at δ 1.96 (1H, *s*, H-12), 1.57-1.62 (1H, *m*, H-13) and δ 1.99-2.02 (1H, *m*, H-2). The multiplet signals between δ 1.06-1.13 and δ 1.48-1.53 could be attributed to H-1.

The ^{13}C NMR spectrum revealed 10 carbon signals as shown in Appendix 14. The DEPT-135 spectrum of **118** indicated the presence of three methyl, one methylene, five methines and one quaternary carbon atoms in a molecule. The methine carbon resonances δ 129.49 and 134.55 indicated the presence of double bonds in the molecule. Based on the spectroscopic data compound **118** was identified as α-phellandrene dimer.

Table 12: ^{1}H and ^{13}C NMR spectral data for **118** and comparison with the data reported for α-phellandrene dimer in the literature, 400 MHz, $CDCl_3$, δ in ppm, *mult*, *J*

C	**118**		α-phellandrene dimer	
	^{1}H NMR	^{13}C NMR	^{1}H NMR	^{13}C NMR
1(10)	1.07-1.13, 1.48-1.53	22.0	1.13-1.18, 1.47-1.51	22.6
2(9)	1.98-2.02	37.4	1.99-2.02	37.7
3(8)	5.58 (*dd*)	129.4	5.73	129.2
4(7)	5.71(*d*)	134.5	5.65	134.8
5(6)		43.4		43.8
11(12)	1.96	37.5	2.03	37.9
13(16)	1.57-1.62	31.8	1.56-1.62	32.1
14(18)	0.92	19.5	0.92	19.6
15(17)	0.90	19.3	0.91	19.4
19(20)	0.94	22.3	1.03	22.6

Comparison of the ^{1}H and ^{13}C NMR data of **118** with those reported in the literature [42, 152] revealed a very close agreement as shown in Table 12. Compound **118** was first isolated from *Canarium luzonicum* by Krua *et al.* in 1986 [152]. However, in the genus *Boswellia,* it was isolated for the first time by Basar in 2005 from *B. frereana* [42].



118

Characterization of epilupeol acetate (163) and α-amyryl acetate (191)

Compounds **163** and **191** were obtained as an inseparable mixture. The mixture gave a violet spot on TLC (ss_1) at *Rf* = 0.70 after visualizing with vanillin/H_2SO_4 reagent. UV-Vis spectrum showed a broad absorption band at 250 nm. The IR spectrum of the mixture showed strong absorption band at 1734 cm^{-1} due to an ester carbonyl and a weak band at 1638 cm^{-1} due to C=C bond.

The ^{1}H NMR spectrum (Appendix 15) showed signals for a mixture of two compounds of which epilupeol acetate (**163**) identified as the major component. The absence of a *dd* (*J* = 4.8 Hz) and the presence of *bt* at δ 4.62 in the signals corresponding to the major component suggested that an acetyl group was present at the *α*-position of C-3, thus confirming the β orientation of the C-3 proton [132]. The olefinic protons resonance appeared at δ 4.70 (1H, *d*, *J* = 2.3 Hz, H-29) and 4.58 (1H, dd, H-29) were assigned for the two methylenic olefinic protons which are characteristic signals for lupane-type skeleton. The spectrum showed the presence of intense eight methyl proton signals at δ 0.80 (*s*, H-25), 0.84 (*s*, H-28), 0.85 (*s*, H-24), 0.88 (*s*, H-27), 1.01 (*s*, H-26), 1.05 (*s*, H-23), 1.69 (*s*, H-30) and 2.09 (*s*, H-32). The one-proton signal observed at δ 2.39 (1H, *td*, *J* = 5.6 and 4.8 Hz, H-19) was assigned for H-19.

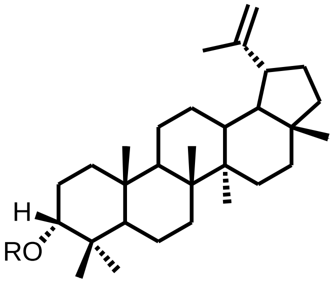
The ^{13}C NMR spectrum of the mixture revealed 32 major carbon signals corresponding to the major component as presented in Appendix 15. The DEPT-135 spectrum indicated eight methyl, eleven methylene, six methine and seven quaternary carbon signals attributed to the major compound. Two carbon resonances were observed δ 109.32 and 151.04 indicating the presence of an exomethylene double bond. The oxygenated carbon signal at δ_C 78.33 was due to acetylated carbon on C-3. The signal due to the ester appeared at δ_C 170.87.

Based on the above discussed spectroscopic data structure **163** was assigned to the major component of the mixture. The identity of **163** as epilupeol acetate was confirmed by comparing the ^{1}H and ^{13}C NMR data of **163** with those reported for epilupeol acetate. Table 13 reveals that there is a very close agreement between the NMR data of **163** and epilupeol acetate isolated from *Cirsium nipponicium* [153, 154].

The minor component of the mixture was identified as α-amyryl acetate (**191**) base on its NMR data. The ration of two compounds in mixture is 1:0.3 based on the peak integration of their olefinic protons. Thus, ^{1}H NMR spectrum of compound **191** revealed the presence of olefinic proton resonance at a δ 5.15 (1H, *t*, *J* = 3.6 Hz, H-12) and an oxymethine proton signal δ 4.65 (1H, *dd, J* = 11.1, 2.8 Hz, H-3). The spectrum further revealed the presence of an acetyl methyl group. The position of the acetyl group must be a C-3 and it must be α-oriented.

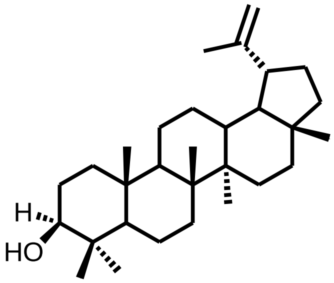
The ^{13}C NMR spectrum revealed olefinic carbon signals at δ 139.51 and 124.47 which are characteristic of an ursane-type skeleton. Furthermore, the typical signal at δ 55.3 attributed to C-5 with the doublet of doublets at δ 4.65 in ^{1}H NMR spectrum confirmed the *α*- orientation of the oxymethine proton attached to C-3. The most downfield signal at δ 171.05 was assigned to the acetyl carbonyl group and the oxygenated carbon signal at δ 80.97 was assigned to C-3 where the acetyl group is attached.

Based on the above spectroscopic data **191** was identified as α-amyryl acetate. The 1H and 13C NMR data of 191 revealed a very close agreement to the data reported for α-amyryl acetate as shown in Table 13. Epi-lupeol acetate and α-amyryl acetate were previously reported from *Gambeya boiviniana* [155]. Epilupeol acetate (**163**) was reported to inhibit the activity of COX-2 [102]. It also exhibited pronounced anti-viral activity against Ranikhet disease virus in chick embryos [156]. Its anti-inflammatory and antioxidant activities were also documented [155]. Likewise α-amyryl acetate (**191**) was reported to exhibit anti-inflammatory [155] and analgesic properties [157].

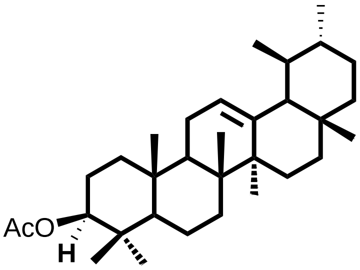


R = H, **158**

R = OAc, **163**



159



191

Characterization of epilupeol (158)

Epilupeol (**158**, BF3) was isolated as a white powder with melting point 202-206 ^{o}C. Analysis by TLC (ss$_1$) showed a strong violet spot at *Rf* = 0.54. The UV spectrum revealed an absorption maximum at 272 nm. The IR absorption bands at 3496 cm^{-1} and 1636 cm^{-1} due to O-H bond and C=C bond vibrations, respectively.

Table 13: ^{1}H and ^{13}C NMR assignments for compounds **158**, **159**, **163**, and **191**, and comparison with the data reported for epilupeol, lupeol, epilupeol acetate and α-amyryl acetate in the literature (400 MHz, $CDCl_3$)

C	**163**		**191**		**158**		**159**	
	Expt.	Lit.[153]	Expt.	Lit [155]	Expt.	Lit.	Expt.	Lit.
1	34.0	33.2	38.39	38.40	33.2	33.2	38.7	38.7
2	25.1	25.3	23.4	23.8	25.4	25.3	27.4	27.4
3	78.3	78.9	80.9	80.8	76.2	76.2	79.0	79.0
4	36.6	37.5	37.8	37.9	37.5	37.5	38.8	38.9
5	50.2	48.9	55.3	55.4	49.0	49.0	55.3	55.5
6	18.1	18.2	18.2	18.3	18.2	18.2	18.3	18.5
7	29.8	29.8	32.7	32.8	34.1	34.1	34.2	34.2
8	41.0	40.9	38.1	38.0	41.0	41.0	40.8	40.9
9	50.2	50.1	47.4	47.6	50.2	50.2	50.4	50.5
10	37.1	37.2	36.8	36.6	37.2	37.2	37.1	37.2
11	20.7	20.7	20.9	21.0	20.7	20.7	20.9	21.0
12	22.9	25.0	124.4	124.2	25.1	25.1	25.1	25.2
13	38.0	37.9	139.5	139.5	38.0	38.0	38.0	38.1
14	42.9	42.8	42.1	42.0	42.9	42.9	42.8	42.9
15	27.4	27.3	26.6	27.5	27.9	27.3	27.4	27.1
16	33.8	35.5	26.1	26.7	35.5	35.5	35.5	35.5
17	43.0	42.9	33.7	33.7	43.0	43.0	43.0	43.0
18	48.0	48.2	59.0	59.0	48.2	48.2	48.3	48.3
19	48.3	48.0	39.7	39.6	48.0	48.0	47.9	48.0
20	150.9	151.0	39.6	39.6	151.0	151.0	150.9	151.0
21	35.6	34.0	31.2	31.2	29.8	29.8	29.8	29.9
22	40.1	39.9	41.5	41.5	40.0	40.0	40.0	40.0
23	19.3	20.2	15.5	14.5	28.2	28.2	28.0	28.0
24	18.0	17.9	28.1	28.0	22.1	22.1	15.3	15.5
25	16.0	16.0	16.5	16.3	15.9	15.9	16.1	16.1
26	15.9	15.8	16.8	16.0	15.9	15.9	15.9	16.0
27	14.7	14.6	19.3	19.3	14.6	14.6	14.5	14.8

28	15.9	15.9	17.5	18.0	18.0	18.0	18.0	18.0
29	109.3	109.2	25.1	25.0	109.3	109.2	109.3	109.0
30	19.3	19.2	19.3	19.4	19.2	19.2	19.3	19.5
31	170.8	170.6	171.0	171.0				
32	21.7	21.1	22.0	21.4				

The ^{1}H NMR spectrum (Appendix 16) of compound **158** showed seven methyl proton signals at δ 0.80 (*s*, H-24), 0.84 (*s*, H-28), 0.86 (*s*, H-25), 0.95 (*s*, H-27), 0.97 (*s*, H-23), 1.05 (*s*, H-26) and 1.70 (*s*, H-30). The multiplet signals at δ 1.48, 1.38-1.43, 1.35 and 1.18-1.27 could be ascribed to methylenic protons. A multiplet at δ 1.94-1.98 indicated the presence of methine protons at C-13 and C-20.

The signal at δ 2.39 (1H, *td*, J = 6 and 4.8 Hz, H-19) could be assigned to the methine proton at C-19 and the broad signal at δ 3.40 (1H, *bt*, J = 2.8 Hz, H-3) was assignable to the proton at the oxygenated carbon C-3. The proton resonance at δ 4.56 (1H, *dd*, J = 2.4, 1.3 Hz, H-29) and 4.70 (1H, *d*, J = 2.0 Hz, H-29) indicated vinylic protons at C-29. The broad triplet at δ 3.40 confirmed the α orientation of hydroxyl group attached to C-3. Thus, the hydrogen atom at C-3 must be β oriented [132].

^{13}C NMR spectrum of **158** showed 30 carbon signals and DEPT-135 spectrum (Appendix 16) confirmed the presence of seven methyl, eleven methylene, six methine and six quaternary carbons. The carbon resonance at δ 109.3 and 151.0 indicated the presence of exomethylene double bond. The signal appeared at δ 76.2 could be attributed to oxymethine carbon C-3.

The above spectroscopic data was compared with previously reported data for epilupeol [158] as shown in Table 13 and a very close agreement was observed. Thus, **158** was identified as epilupeol. It was first isolated from *B. frereana* in 1981 by Proietti [51] and from *B. sacra* in 2011 by Morikawa [78].

Characterization of lupeol (159)

Lupeol (**159**, BF4), $C_{30}H_{50}O$, was obtained as a white crystal with melting point range 214-217 ^{o}C (215-216 ^{o}C [159]). A violet spot was observed on TLC at *Rf* = 0.43 (ss_1). The UV-Vis spectrum showed absorption maximum at 272 and 320nm. The EIMS showed a molecular ion peak at *m/z* 426 and relevant fragment ions at *m/z* 189 and 207, which are considered to be characteristic for the fragmentation of triterpenes with a lupane skeleton bearing a hydroxyl group at C-3 [160].

The IR and NMR data of compound **159** closely resembled those of **158** discussed above with few exceptions. The oxymethine proton signal appeared as a doublet of doublets at δ 3.20 (1H, *dd*, *J* = 11.2, 5.0 Hz, H-3) and this splitting pattern has been used to differentiate between lupeol and epilupeol (δ 3.40, *t*, H-3) [132]. The oxymethine carbon signal in the ^{13}C NMR spectrum of **159** appeared at δ 79.0 and is more deshielded than the C-3 signal in **158** (δ 76.0). Moreover, the C-5 resonance appeared at δ 55.3 in the spectrum of **159** which is more deshilded than the corresponding signal in **158** (δ 49.0). Thus, the OH group at C-3 in **159** must be β-oriented.

Based on the above spectroscopic data, it was evident that **159** was the C-3 epimer of **158.** Thus, **159** was identified as lupeol. Comparison of the 1H and 13C NMR data of 159 with those reported for lupeol by Abdullahi in 2013 [161] showed a very close agreement (Table 13). Lupeol was first reported in 1983 from *Plumeria obtusifolza* [162].

The biological activities of epilupeol (**158**) and lupeol (**159**) were studied and anti-microbial and anti-fungal activities against *Fusarium oxysporum* and *Penicillium notatum* [158, 163] were recorded. These compounds exhibit anti-inflammatory activity [102, 163], anti-diabetic activity [164], prevent collagen degradation and inhibit the production of pro-inflammatory mediators and matrix metalloproteinase [100].

2.2.5. Compounds isolated from *B. pirottae* resin

The ethanol extract of *B. pirottae* showed four major spots on TLC (ss_1, *Fig. 10, 13a and 13b*). Thus TLC pattern was found to be similar to what was observed for the extract of the resin of *B. neglecta*. Column chromatography separation of the crude extract gave four components, BPi2 (*Rf* = 0.72), BPi3 (*Rf* = 0.62), BPi4 (*Rf* = 0.54) and BPi5 (*Rf* = 0.43). Surprisingly all four components turned out to be mixtures of compounds. NMR studies of the mixtures helped to identify the constituents as a mixture of triterpenes as described below.

All mixtures of triterpenes gave single spots on TLC in various solvent systems and all attempts to separate the mixtures in to their constituent parts were unsuccessful. A total of 19 compounds including eight fatty acid esters of triterpenes and eleven triterpenes were identified. The ^{1}H and ^{13}C NMR spectra of BPi2 revealed that it consisted eight compounds. These were identified to be fatty acid esters of *epi*-lupeol, lupeol, α-amyrin, β-amyrin and tirucall-8, 24-dien-3α-ol. BPi3 consisted of a mixture of four compounds (**129**, **148**, **157** and **200**). BPi4 were found to be a mixture of three compounds (**130**, **149** and **201**) and BPi5 consisted of four compounds (**131**, **150**, **159** and **170**).

TLC of the EtOH extracts of the leaves and bark of *B. pirottae* were compared with the extract of the resin and were found to be comparable. To the best of our knowledge, this is the first report on the isolation and identification of the aforementioned compounds from *B. pirottae*.

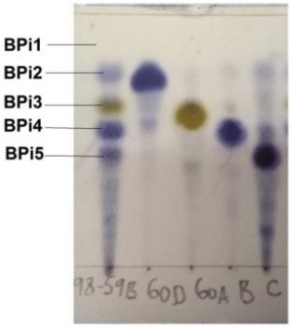


Figure 10: TLC chromatogram of the EtOH extract of the resin of B. pirottae and isolated compounds (ss_1)

Characterization of BPi2 (compounds 192-199)

BPi2 was obtained as a yellow oil and a single violet spot was observed on TLC (ss_1) at *Rf* = 0.72. 1H and 13C NMR spectra of Bpi2 revealed that it was composed of a mixture of eight compounds. Even though it was difficult to estimate the relative amounts of the components by integrating the olefinic and oxymethine proton resonances, it was possible to identified four different skeletons from the olefinic carbon resonances in the ^{13}C NMR spectrum Bpi2 (Table 14, Appendix 18).

The double bond signals δ 139.6, 139.5, 139.5, 124.6, 124.6 and 124.3 could be attributed to three urs-12-en-type skeletons (**192-194**) and the signals δ 145.3, 145.0, 121.9 and 121.8 were indicative for two olean-12-ene-type skeletons (**195** and **196**). Furthermore, the olefinic carbon signals at δ 125.2, 130.9, 133.6 and 133.9 were assigned to tirucallane type (**199**) skeleton while the signals at δ 151.0, 150.8 and two 109.3 revealed the presence of two lupane-type skeletons (**197** and **198**). The appearance of eight carbonyl carbon signals between δ173.8-174.3 suggested the presence of long chain fatty acid esters. The absence of carbonyl carbon signals between δ 170.0-171.5 was indicative of the absence of acetylated derivatives of the triterpenes in the mixture.

On the other hand, the eight oxygenated carbon signals in between δ 80.7- 78.0 were assignable for oxymethine carbons C-3 where the ester groups were attached. The presence of signals for eight terminal methyl groups in the most upfield region between δ 14.1 and 14.7 and the absence of additional olefinic carbon signals in the olefinic region was suggestive of the saturated fatty acid esters.

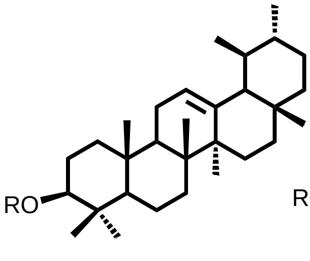
The IR spectrum of the Bpi2 showed a medium absorption band at 1735 cm^{-1} due to the presence of ester carbonyl carbons and band at 1638 cm^{-1} indicated the presence of C=C bonds.

The ^{1}H NMR spectrum of Bpi2 exhibited oxymethine protons signal at δ 4.49 (*dd*, *J* = 4.0 and 7.6 Hz) corresponding to the fatty acid esters of α- amyrin, β-amyrin, and 3β-OH-tirucallol. On the other hand, the oxymethine proton signal at δ 4.65 (*bt*) attributed to the fatty acid ester of epi-lupeol. The two olefinic proton signals at δ 4.70 (*d*, *J* = 1.2 Hz) and 4.59 (*bs*) indicated the presence of lupeol-type fatty acid esters. The most downfield olefinic proton signals appeared as triplets at δ 5.12, 5.17 and 5.21 and were suggestive of the presence of tirucallane-, ursane- and oleanane-type fatty acid esters, respectively.

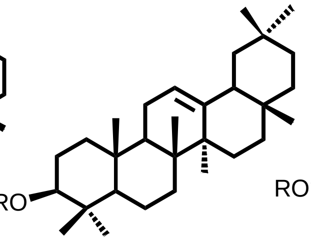
^{1}H and ^{13}C NMR data (Table 14) allowed for the identification of compounds 192-199 in Bpi2. The characteristic ^{1}H and ^{13}C data were compared with those reported for 192-199 in the literature [155, 165] and close agreement were observed. The effort made to estimate the numbers of carbon atoms on the side chains from GC-MS spectroscopic data was unsuccessful.

Table 14: NMR data of fatty acid esters triterpenes isolated from *B. pirottae (*400 MHz, $CDCl_3$)

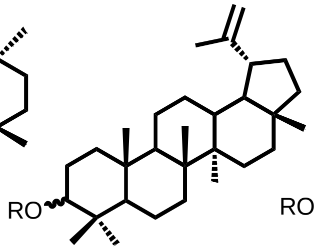
Carbons	**192**	**193**	**194**	**195**	**196**	**197**	**198**	**199**
3	80.7	80.7	80.7	80.7	80.7	78.0	78.0	78.1
8								133.6
9								133.9
12	124.3	124.6	124.6	121.8	121.9			
13	139.6	139.5	139.5	145.0	145.3			
20						109.3	109.4	
24								125.2
25								130.9
29						150.8	150.9	
1’	173.8	173.8	173.9	173.4	174.4	174.2	174.2	174.3



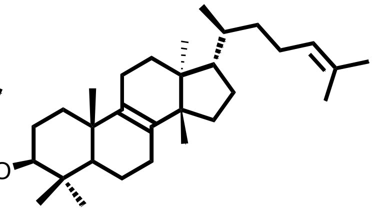
192-194



195-196



197-198



199

R= $CH_3(CH_2)nCO^-$, n>0

The existence of lupeol fatty acid ester, α-amyrin fatty acid ester, β-amyrin fatty acid ester as a mixture has been reported previously from *Artocarpus ovatus* and *Gambeya boiviniana* [157, 166, 167].

Characterization of α-amyrone, β-amyrone, lupenone and tirucall-8, 24-dienone

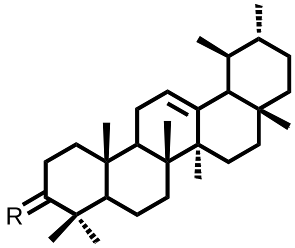
BPi3 was obtained as yellow oil. It gave a yellow spot on TLC (ss_1), at *Rf* = 0.62, which on heating changed to deep blue color.

The 1H NMR showed that Bpi2 was a mixture of four compounds in a relative ratio of 1:0.7:0.4:0.2 and were presumed to be the pentacyclic triterpenes **129**, **148** and **157** and the tetracyclic triterpene **200**. The IR spectrum of Bpi2 showed strong absorption band at 1713 cm^{-1} due to carbonyl groups. The absence of two medium bands at 2725-2827 cm^{-1}, ruled out the presence of aldehyde carbonyl groups. The band at 1638 cm^{-1} revealed the presence C=C bonds.

In the ^1H NMR spectrum of BPi3 (Appendix 19), five olefinic proton signals were observed. Three proton signals at δ 5.19 (*t, J* = 3.6 Hz, H-12), 5.14 (*t, J* = 3.2 Hz, H-12) and 5.08 (*t, J* = 6.8 Hz, H-24) were assigned to the olefinic protons of compound **129**, **148** and **200**, respectively, whereas two proton signals at δ 4.67 (*d*, J = 2.0 Hz) and 4.55 (b*s*) were assigned to the geminal olefinic protons of compound **157**. Multiplets appeared at δ 2.34-2.42 which were assigned to protons on C-2 α to carbonyl carbons. The lack of proton signals at δ 9.0-10.0 confirmed the absence of aldehyde protons in with the IR observation.

The ^{13}C NMR spectrum of Bpi3 (Appendix 19) revealed typical signals at δ 217.95, 217.91, 217.61 and 217.56 corresponding to the carbonyl carbons of **157**, **200**, **129** and **148**, respectively. The olefinic carbon signals at δ 109.51 and 150.80 (quaternary) can be attributed to **157.** The signals at δ 121.51 and 145.22 were assigned to compound **148** and the signals at δ 139.70 and 124.19 corresponded to compound **129**. The remained olefinic carbon signals at δ 134.72, 123.65, 130.84 and 125.20 were due to compound **200**. Comparison of the ^{13}C NMR data of compound **129**, **148**, **157** and **200** were compared with those reported in the literature [167-170] for α-amyrone, β-amyrone, lupenone, tirucall-8, 24-dienone, respectively, showed very close agreements as shown in Table 15.

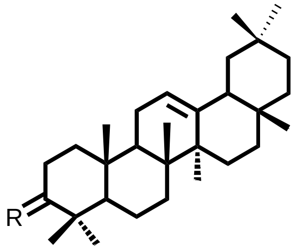
The common appearances of **129** and **148** in *Protium heptaphyllum* [167], **148** and **157** in *Corynaea crassa* [168], and **129**, **148** and **157** in *Rourea doniana* [169] were reported. The common appearance of these four triterpenes in the genus *Boswellia* has not been reported before.



R = O, **129**

R = α–OH, 130

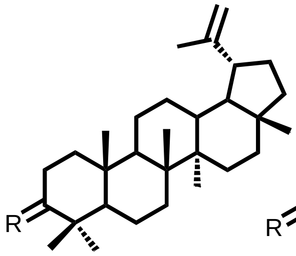
R = β-OH, **131**



R = O, **148**

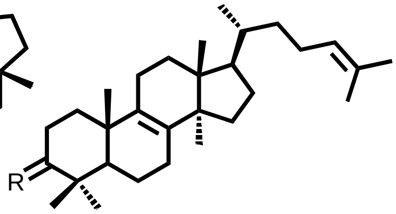
R = α–OH, 149

R = β-OH, **150**



R = O, **157**

R = β-OH, **159**



R = O, **200**

R = α–OH, **201**

R = β-OH, **170**

Characterization of *epi-α*-amyrin, *epi*-β-amyrin and 3α-OH-tirucall-8, 24-diene (130, 149 and 201)

BPi4 was obtained as yellow oil. TLC revealed a spot at *Rf* = 0.54 (*ss₁*) which turned deep violet after heating on the TLC plate. Both ^{1}H and ^{13}C NMR data revealed that Bpi4 was a mixture the triterpenes as **130**, **149** and **201** as documented below. Integration of the olefinic proton signals in the ^{1}H NMR spectrum of mixture was used to estimate the relative ratio of the three compounds as 1.0: 0.4: 0.2.

The IR spectrum of Bpi4 showed an absorption band at 3428 cm^{-1} confirming the presence of O-H groups. The weak band at 1638 cm^{-1} was due to C=C bond vibration. Another characteristic band at 1375 cm^{-1} suggested secondary C-O bond stretching and the weak band at 1074 cm^{-1} was due to C-O bending.

Table 15: ^{13}C NMR spectral data of **148**, **129**, **157** and **201**, and comparison with the data reported for α-amyrone, β-amyrone, lupenone and tirucall-8,24-dienone in the literature (400 MHz, CDCl$_3$)

CN	**148**		**129**		**157**		**200**	
	Expt.	Lit. [167]	Expt.	Lit. [168]	Expt.	Lit. [169]	Expt.	Lit. [170]
1	39.3	39.2	39.6	39.6	39.6	39.6	36.3	36.1
2	26.4	26.3	26.6	26.6	34.1	34.1	34.1	34.3
3	217.5	217.8	217.6	217.2	217.9	218.2	217.9	217.7

4	47.4	47.4	47.3	47.5	47.3	47.3	47.4	47.4
5	55.3	55.2	55.2	55.3	54.9	54.9	50.1	50.9
6	19.6	19.2	19.6	19.7	19.3	19.2	19.7	19.4
7	32.4	32.4	34.2	34.3	33.5	33.5	28.4	28.3
8	39.7	39.3	40.0	40.1	40.7	40.7	132.6	132.2
9	47.2	47.1	46.9	46.9	49.7	49.7	134.7	133.8
10	36.6	36.5	36.6	36.7	36.8	36.8	36.6	36.9
11	23.6	23.6	23.5	23.6	21.4	21.4	21.2	21.1
12	121.5	121.2	124.2	124.2	25.1	25.1	26.6	26.3
13	145.2	145.2	139.7	139.7	38.1	38.1	44.1	44.6
14	41.8	41.8	42.2	42.3	42.8	42.8	50.0	50.0
15	26.1	26.0	28.1	28.1	27.4	27.4	31.0	31.0
16	26.9	26.8	26.6	26.6	35.5	35.5	30.7	30.9
17	32.1	32.1	33.8	33.8	42.9	42.9	51.4	51.3
18	46.8	46.8	59.1	59.2	48.2	48.2	18.6	18.1
19	46.7	46.7	39.7	39.7	47.9	47.9	20.2	19.7
20	34.5	35.0	39.7	39.7	150.7	150.8	33.3	33.1
21	34.7	34.6	31.2	31.3	29.8	29.6	18.6	18.7
22	37.1	37.0	41.5	41.6	39.4	39.4	44.1	44.4
23	28.0	28.4	28.8	28.8	26.6	26.6	26.7	26.8
24	15.2	15.1	21.5	21.5	21.0	21.0	125.2	125.3
25	15.4	15.4	15.4	15.5	15.9	15.9	130.8	130.9
26	16.7	16.7	16.8	16.9	15.8	15.7	24.2	24.2
27	25.8	25.8	23.2	23.2	14.5	14.4	26.6	26.2
28	26.1	26.1	26.6	26.6	18.0	17.9	25.7	25.7
29	34.1	34.1	17.5	17.5	109.4	109.3	15.5	15.9
30	23.6	23.6	21.5	21.4	19.6	19.6	21.3	21.3

The ^{1}H NMR spectrum of BPi4 (Appendix 20) showed methyl proton resonances at δ 0.79-1.10. A typical signals at δ 5.19 (*t, J* = 3.2 Hz, H-12), 5.14 (*t, J* = 3.6 Hz, H-12) and

5.11 (*t, J* = 7.2 Hz, H-24) were assigned to olefinic protons of **130, 149**, and **201**, respectively. Additionally, the signals due to oxymethine protons of these isomeric triterpenes were observed as a broad singlet at δ 3.42. The fact that the oxymethine proton signals appeared as singlet is indicative of the α-orientation of the hydroxyl groups at C-3 [132].

The ^{13}C NMR spectrum (Appendix 20) of BPi4 was used to confirm the identities of **130**, **149** and **201**. The chemical shifts of the olefinic carbons were compatible with the presence of triterpenes **130** (δ 145.1 and 121.7), **149** (δ 139.5 and 124.4) and **201** (δ 134.3, 133.3, 130.8 and 125.2) triterpenes. The characteristic oxymethine carbon resonance at δ 76.1 was assigned to **130** and **149**, while the signals δ 75.9 was attributed to compound **201**.

Comparison of NMR data of **130**, **149** and **201** with those reported for *epi-α*-amyrin, *epi-β*-amyrin and *3α*-OH-tirucall-8, 24-diene in the mixture, respectively, [171] revealed a very good agreement as shown in the Table 16.

Characterization of *α*-amyrin (131), β-amyrin (150), lupeol (159) and 3β-OH-tirucall-8, 24-diene (170)

BPi5 was obtained as yellow oil and a violet spot was observed on TLC, at *Rf* = 0.43 (*ss1*). Both ^{1}H and ^{13}C NMR data revealed that Bpi5 was a mixture of four triterpenes. Integration of olefinic proton resonance in the ^{1}H NMR spectrum of Bpi5 revealed relative ratio of 1.0: 0.4: 0.4: 0.3 for compounds **131, 159, 170** and **150**, respectively. The IR spectrum of Bpi5 exhibited a very intense absorption band at 3440 cm^{-1} due to a hydroxyl groups and a medium absorption band at 1638 cm^{-1} corresponding to C=C bonds.

In the ^{1}H NMR spectrum (Appendix 21) of Bpi5, the olefinic proton resonances appeared at δ 5.20 (*t, J* = 3.6, H-12), 5.14 (*t, J* = 3.6, H-12) and 5.10 (*t, J* = 7.2, H-24) and were assigned to compounds **131, 150** and **170**, respectively. The signals at δ 4.70 (*d*, *J* = 1.6 Hz, H-29) and 4.58 (*dd, J* = 2.4, 1.3 Hz, H-29) were assigned to the olefinic methylene protons of compound **159**. In addition, typical oxymethine proton resonances were appeared at δ 3.26 (*dd*, *J* = 11.2, 5.3 Hz, 1H), and 3.20 (*dd*, *J* = 11.0, 4.7 Hz, 1H) which indicated that the hydroxyl groups at C-3 should have β orientation, which differentiate these triterpenes from their corresponding *epimers*.

As shown in Appendix 21, the ^{13}C NMR spectrum revealed typical olefinic carbon signals at δ 109.3 and 150.9 attributed to compound **159**. Likewise, the signals at δ 145.2 and 121.7 can be assigned to compound **150** and the signals at δ 139.6 and 124.4 correspond to compound **131**. The remaining olefinic carbon resonances at δ 134.0, 133.5, 130.9 and 125.0 are due to compound **170**. The other characteristic oxygenated carbon signal at δ 79.06 was assigned to compounds **131** and **150**, whereas, the signal at δ 79.01 was attributed to compounds **159** and **170**.

Table 16: ^{13}C NMR spectral data of **130**, **131**, **149**, **150**, **170** and **201** and comparison of epi-α-amyrin, α-amyrin, *epi*-β-amyrin, β-amyrin, 3β-OH-tirucall-8, 24-diene and 3α-OH-tirucall-8, 24-diene (400 MHz, $CDCl_3$)

CN	**130**		**149**		**201**		**131**		**150**		**170**	
	Expt.	[171]	Expt.	[171]	Expt.	[172]	Expt.	[31]	Expt.	[31]	Expt.	[168]
1	33.2	33.3	33.0	33.0	30.8	31.0	38.8	38.8	38.6	38.5	35.2	35.2
2	26.6	26.6	25.2	25.3	25.8	25.9	27.3	27.3	27.3	27.2	27.9	27.9
3	76.1	76.2	76.1	76.1	75.9	76.0	79.0	79.0	79.0	79.0	79.0	79.0
4	37.3	37.4	37.3	37.3	37.6	37.7	38.8	38.8	38.7	38.7	38.9	38.9
5	48.9	48.9	48.9	48.9	44.8	44.9	55.2	55.3	55.2	55.1	50.9	50.9
6	18.3	18.4	18.7	18.3	18.8	18.8	18.3	18.4	18.6	18.3	18.9	18.9
7	32.8	32.8	32.5	32.5	26.1	26.2	32.9	33.0	32.6	32.6	27.6	27.6
8	40.2	40.2	39.9	39.9	133.3	133.2	40.0	40.0	39.8	39.7	133.5	133.5
9	47.5	47.6	47.4	47.3	134.3	134.5	47.7	47.8	47.6	47.6	134.0	134.0
10	37.9	37.0	37.0	37.0	36.1	37.3	36.9	36.9	36.9	36.9	37.2	37.2
11	23.2	23.3	23.4	23.4	21.4	21.4	23.4	23.4	23.5	23.5	21.4	21.4
12	124.5	124.5	121.7	121.7	29.9	29.9	124.4	124.3	121.7	121.7	29.8	29.8
13	139.5	139.6	145.1	145.1	44.4	44.4	139.6	139.6	145.2	145.1	44.1	44.1
14	42.2	42.2	41.8	41.7	50.1	50.0	42.1	42.2	41.7	41.7	49.9	49.9
15	28.1	28.2	26.1	26.1	27.2	27.3	28.1	28.2	26.1	26.1	30.8	30.7
16	25.2	25.3	26.9	26.9	28.1	28.1	26.6	26.7	26.9	26.9	28.0	28.0

17	33.7	33.8	32.5	32.4	46.8	46.4	33.7	33.8	32.5	32.4	50.1	50.1
18	59.1	59.2	47.2	47.2	15.6	16.1	59.0	59.1	47.2	47.2	20.1	20.1
19	39.6	39.7	46.8	46.8	19.9	20.0	39.6	39.7	46.8	46.8	15.4	15.4
20	39.6	39.7	31.1	31.0	50.0	49.0	39.6	39.7	31.3	31.0	36.3	36.3
21	31.3	31.3	34.7	34.7	36.3	176.8	31.2	31.3	34.3	34.7	18.7	18.6
22	41.5	41.6	37.1	37.1	31.3	31.7	41.5	41.6	37.1	37.1	36.4	36.4
23	28.7	28.8	28.3	28.2	26.1	26.6	28.1	28.1	28.0	28.0	24.9	24.9
24	22.3	22.4	22.2	22.3	125.8	123.8	15.7	15.7	15.5	15.5	125.2	125.2
25	15.4	15.5	15.3	15.2	130.5	132.6	15.6	15.7	15.4	15.4	130.9	130.9
26	16.8	16.9	16.8	16.8	17.6	17.7	16.8	16.9	16.8	16.8	25.7	25.7
27	23.4	23.4	26.0	26.0	25.7	25.7	23.3	23.3	26.0	25.9	17.6	17.6
28	28.3	28.4	28.4	28.4	29.7	29.1	28.0	28.1	28.4	28.3	17.6	17.6
29	17.4	17.5	33.3	33.3	22.2	22.3	17.5	17.5	33.3	33.3	28.0	28.0
30	21.4	21.4	23.7	23.6	24.4	24.5	21.4	21.5	23.5	23.5	24.3	24.3

Based on the above spectroscopic data compounds **131, 150, 170** and **159** were identified as α-amyrin (*3β*-hydroxy-urs-12-en-3-ol), β-amyrin (3β-hydroxy-olean-12-en-3-ol), 3β-OH-tirucall-8, 24-diene and lupeol, respectively. Comparison of the NMR data of **131, 150, 170** and **159** with those reported in the literature gave good agreements as shown in Table 16 [24, 70, 173].

2.2.6. Compounds isolated from *B. neglecta* resin

As mentioned in Section 2.3.5, the TLC (ss_1, *Fig. 11, 13a* and *13b*) profile of the EtOH extract of *B. neglecta* resin closely resembled the TLC profile of *B. pirottae*. Particularly, the appearance of a yellow spot on TLC while spraying with vanillin/H_2SO_4 that changed immediately to deep violet color on heating and gradually changed to blue at room temperature was characteristic to the extracts of both resins.

From the neutral fraction of extract of *B. neglecta* resin (BnRn), three major fractions were isolated by silica gel column chromatography. Although each fraction gave a single spot on TLC, BN2 (*Rf* = 0.62), BN3 (*Rf* = 0.54) and BN4 (*Rf* = 0.43), spectroscopic studies revealed that each was a mixture of compounds. Thus, BN2 consisted of three compounds (**129**, **148** and **157**), BN3 consisted of three compounds (**130**, **149** and **158**) and BN4 consisted of four compounds (**131**, **150**, **159** and **202**).

Comparison of the chemical profile of the resin of *B. neglecta* with that of the resin of *B. pirottae* revealed some difference. The presence of *epi*-lupeol (**158**) and *3β*-OH-tirucall-7,24-diene (**202**), and absence of 3-oxo-tirucallene (**200**), 3α-and 3β-tirucallan-8,24-dienol (**201** and **170**, respectively) in the resin of *B. neglecta* differentiate this resin from *B. pirottae*. Moreover, fatty acid esters of triterpenes were not detected in the resin of *B. neglecta* in this study.

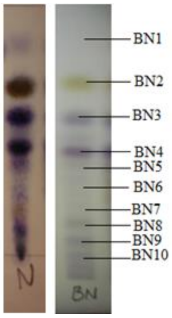


Figure 11: TLC chromatogram of EtOH extract of *B. neglecta* (ss$_1$)

(a: EtOH extract, b: neutral fraction*, ss$_1$*)

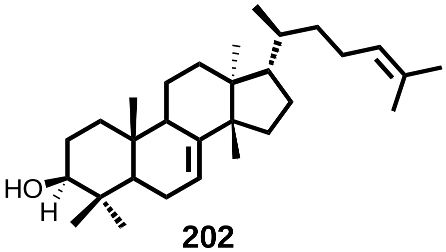
Characterization of *3β*-OH-tirucall-7,24-diene (202)

3β-OH-Tirucall-7,24-diene (**202**) was found to be one of the constituent of BN4 together with **131**, **150** and **158**. The ^{1}H NMR spectrum (Appendix 22) of BN4 revealed the presence of six olefinic proton resonances of which two for **159** (δ 4.5 and 4.7), one for **131** (δ 5.14), one for **150** (δ 5.19) and two for **202** (δ 5.11 and 5.27). **31**, **150** and **159** were identified as α-amyrin, β-amyrin and lupeol, respectively, as described above.

The signal at δ 5.27 which appeared as triplet is typical of 202 and was assigned to olefinic proton attached to C-7. The other olefinic proton resonances at δ 5.11 also appeared as a triplet and was assigned to the proton at C-24 as described above for **170** and **201**.

The ^{13}C NMR spectrum (Appendix 22) of BN4 showed olefinic carbon signals at δ 117.79, 125.14, 130.92 and 145.82 that can be attributed to compound **210**. The carbon signal at δ 117.79 together with olefinic proton resonance at δ 5.27 indicated that one of the double bonds is in the ring system of **202** and found between C-7 and C-8 in the tirucallane skeleton. Thus, the olefinic carbon resonances at δ125.14 and 130.92 must be attributed to C-24 and C-25 on the side chain. The oxymethine carbon signal at δ 79.2 suggested that the OH group at C-3 had a β-orientation [132].

Table 17 gives the 13C NMR data of **202**. Based on this, **202** was identified to be 3-β-OH-tirucall-7,24-diene. Comparison of the NMR data of **202** with those reported for 3-β-OH-tirucall-7,24-diene [174] revealed a very close agreement.



202

Table 17: ^{13}C NMR spectral data for **202** and comparison with the data reported for 3-β-OH-tirucall-7,24-diene (400 MHz, $CDCl_3$)

C	Expt.	[174]	C	Expt.	Lit. [174]	C	Expt.	Lit.
1	37.1	37.1	11	27.2	27.2	21	18.3	18.1
2	27.6	27.6	12	33.9	34.0	22	35.9	36.1
3	79.2	79.0	13	43.5	43.4	23	24.9	25.0
4	38.8	38.8	14	51.9	51.0	24	125.1	124.7
5	50.6	50.5	15	33.8	33.8	25	130.9	130.3
6	23.9	23.9	16	28.4	28.1	26	25.7	25.6
7	117.7	117.3	17	53.2	52.8	27	17.6	17.6
8	145.8	145.3	18	13.1	13.1	28	22.1	21.8
9	48.9	48.9	19	18.3	18.1	29	27.7	27.6
10	35.8	35.8	20	35.9	36.1	30	14.7	14.3

2.2.7. Compounds isolated from *B. rivae* resin

The EtOH extract of the resin of *B. rivae* showed several spots on TLC (*Fig. 12, 13a* and *13b*) after visualizing spots with vanillin/H_2SO_4 reagent. This yellowish jelly extract was subjected to normal phase column chromatography on silica gel to afford six compounds, i. e, (20S)-20-hydroxydammar-24-en-diol acetate (**203**, BR2), 20S,24S-epoxydammarane-25-ol-yl acetate (**204**, BR3), (20S)-20-hydroxydammar-24-en-3-one (**205**, BR4), 20S,24S-epoxydammaran-25-ol-3-one (**206**, BR5), and a mixture of dammaranelactone-3α-acetate (**207**, BR6) and (3β,20S)-dammar-24-endiol (**117**, BR7) as described below.

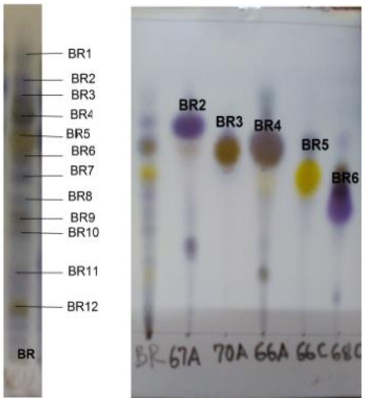


Figure 12: TLC chromatogram of crude extracts and isolated compounds (ss_1)

Characterization of 20-hydroxydammar-24-en-diol acetate (203)

20-Hydroxydammar-24-en-diol acetate (**203**, BR2) was isolated as a colorless oil. It showed a violet spot on TLC (ss_1) at *Rf* = 0.67. The UV spectrum showed an absorption band at 238.5 nm in EtOH. The IR spectrum revealed bands at 3465, 1713 and 1638 cm^{-1} due to OH, ester carbonyl and C=C vibrations.

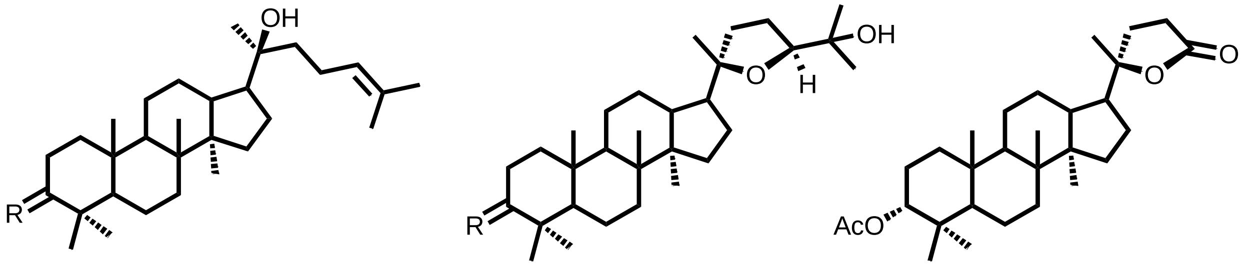
The ^1H NMR spectrum (Appendix 23) of **203** showed nine methyl proton singlet at δ 0.88 (H-30), 0.94 (H-18), 0.98 (H-29), 1.16 (H-28), 1.70 (H-26), 1.18 (H-19), 1.23 (H-21), 1.64 (H-27), and 2.10 (H-32). The singlet at δ 2.10 is due to an acetyl methyl group. The signal at δ 5.14 (*t, J* = 6 Hz, H-24) was indicative of the presence of one double bond in the molecule. The broad singlet at δ_C 4.64 was suggestive of a proton attached to the oxygenated carbon at C-3 (H-3) with β-orientation relative to the electron withdrawing acetyl group.

The ^{13}C NMR spectrum together with the DEPT-135 spectrum revealed 32 carbon signals (Appendix 23) representing nine methyl, ten methylene, six methine and seven quaternary carbons. Two olefinic carbon signals appeared at δ 124.7 and 131.6 which were indicative of the presence of a double bond in dammarane-type triterpenes, where the double bond is found at C-24 and a hydroxyl group at C-20.

The oxygenated methine (δ 78.4) and quaternary carbon (δ 75.4) were attributed to C-3 and C-20, respectively. The most downfield signal at δ 170.8 is due to an acetyl carbonyl carbon. The C-1 and C-5 resonances appeared at δ 34.2 and 50.4, respectively, and hence the acetyl group was placed in the α-orientation. If the orientation of the acetyl group was β, the C-1 and C-5 resonances would have appeared more downfield than the above signals due to the deshielding effect of acetyl group.

It was previously shown [175] that the stereochemistry of C-20 can be easily distinguished either R or S by the chemical shifts of C-20, C-21 and C-22 [175]. In case of C-(20S) the C-21 resonance was found to be more deshielded than that of the corresponding compound with R configuration at C-20, whereas, the chemical shift of C-22 of the C-20(S) compound is more shielded than that of its C-20(R) counterpart [176]. Moreover, the chemical shift of the oxymethine carbon (C-20) helps to distinguish the R and S isomers at C-20. It was found out that the chemical shift of the S isomer was found to appear up field compared to the R isomer [175]. Thus, the stereochemistry of C-20 in compound **203** is S.

The NMR data discussed above was found to resemble the NMR data reported for (20S)-20-hydroxydammar-24-en-diol acetate reported by Tori in 1988 [8, 177]. A close comparison of 13C NMR data as shown in Table 18 helped to conclude that compound **203** was identical to (20S)-20-hydroxydammar-24-en-diol acetate.



R = β-OH, **177**

R = α-OAc, **203**

R = O, **205**

R = α-OAc, **204**

R = O, **206**

207

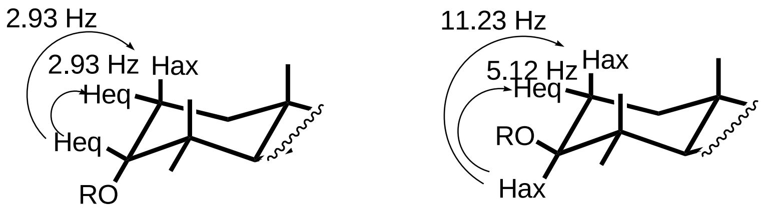
Characterization of 20S, 24S-epoxydammarane-25-ol-yl acetate (204)

Compound **204** (BR3) was isolated as a colorless oil. The UV spectrum showed an absorption maximum at 241nm in EtOH. TLC showed a spot at *Rf* = 0.61 which turned yellow after the TLC plate was treated with vanillin/H_2SO_4 reagent and heated. The same spot turned green upon cooling to room temperature. The compound displayed an IR absorption band at 1726 cm^{-1} due to an ester moiety and a medium band at 3465 cm^{-1} due to O-H group.

The ^{1}H NMR spectrum of **204** (Appendix 24) disclosed nine singlets at δ 0.83 (H-28), 0.87 (H-19), 0.88 (H-29), 0.92 (H-30), 0.98 (H-18), 1.12(H-27), 1.15 (H-21), 1.19 (H-26) and 2.08 (H-32) due to the methyl protons. The doublet of doublets at δ 3.63 (*J* = 5.6 and 4.4 Hz) and the triplet at δ 4.62 (*J* = 2.8 Hz) were suggestive of the presence of two oxymethine protons assigned to protons on C-24 and C-3, respectively. The β-orientation of H-3 could be inferred from the chemical shift of the triplet signal at δ 4.62.

The stereochemistry C-3 can be demonstrated based on the literature data previously reported [178] for similar compounds as follows. The chemical shifts for 3-C and C-29 of α- orientation of the group attached at C-3 differed remarkably from those β-orientation. In case of the α-isomer, the chemical shift of C-3 and C-29 would be appeared at 78.4 and 21.7, respectively. On the other hand, the corresponding signals of β-isomer were observed at 81.0 and 15.4. Moreover, the chemical shift of H-3 helpful to justify the stereochemistry of C-3. When H-3 in α-isomer is equatorial, coupling constants (J2ax-3 and J2eq-3) were almost equal to each other (2.93 Hz), therefore this signal (due to H-

3) looked like triplet. As H-3 of β-isomer is axial, the J2ax-3 and J2eq-3 were quite different (11.23 and 5.12, respectively). Therefore the signal due to H-3 would be clearly doublet of doublet.



The ^{13}C NMR spectrum of **204** revealed 32 carbon signals as presented in Table 18 and Appendix 24. The DEPT-135 spectrum indicated the presence of nine methyls, ten methylene, six methine and seven quaternary carbon atoms. Four oxygenated carbon resonances at δ 70.2, 78.3, 86.3 and 86.5 and were assigned to C-25, C-3, C-24, and C-20, respectively. The presence of an acetyl moiety was confirmed by the carbonyl signals at δ 170.7.

The spectroscopic data generated for **204** was good agreement with previously reported data for 20S,24S-epoxydammarane-25-ol-yl acetate. Table16 gives a comparison of the 13C NMR data of **204** with the data reported for 20S,24S-epoxydammarane-25-ol-yl acetate [179]. Compound **204** was first isolated in 1965 from *Fozrqwieria splendens* [180]. It was reported to have significant cell growth inhibitory activity.

Characterization of Hydroxy-dammarenone II (205)

Compound **205** (BR4) was isolated as a colorless oil. It gave violet spot on TLC (*ss1*) at *Rf* = 0.58 and its UV spectrum gave an absorption maximum 239 nm. The IR spectrum of **205** disclosed a medium band at 3465 cm^{-1} indicating the presence of an O-H group. The strong band at 1700 cm^{-1} suggested the presence of a ketone functional group. The weak band at 1650 cm^{-1} was due to C=C bond stretching.

The ^{1}H NMR spectrum (Appendix 25) showed eight singlets at δ 0.89 (H-30), 0.95 (H-18), 1.01 (H-19), 1.04 (H-29), 1.09 (H-28), 1.16 (H-21), 1.63 (H-27) and 1.70 (H-32) due to methyl group. The multiplets between δ 2.39-2.55 were due to protons on C-2 and C-1 close to the carbonyl carbon C-3. The triplet at δ 5.13 (*J* = 7.2 Hz) was assigned to an olefinic proton at C-24 (H-24). The ^{13}C NMR spectrum revealed 30 carbon signals corresponding to eight methyl, ten methylene, five methine and seven quaternary carbon atoms (Appendix 25). The signal δ 218.1 and the two olefinic carbon signals at δ 124.6 and 131.6 proved the presence of a ketone functional group and one double bond in the molecule. The signal at δ 75.3 was indicative of the presence of an oxymethine carbon at C-20.

It was evidenced from ^{1}H and ^{13}C NMR data discussed above that compound **205** was a hydroxydammarenone II ((20S)-20-hydroxydammar-24-en-3-one) [176, 181] (Table 15). The stereochemistry of the hydroxyl group at C-20 was predicted the same way compound **203**.

Table 18: ^{13}C NMR spectral data of compound **203**-**207** and **177**, and comparison with the data for (20S)-20-hydroxydammar-24-en-diol acetate, 20S,24S-epoxydammarane-25-ol-yl acetate, (20S)-20-hydroxydammar-24-en-3-one, 20S,24S-epoxydammaran-25-ol-3-one, dammaranelactone-3α-acetate and (3β,20S)-dammar-24-endiol (400 MHz, CDCl$_3$)

C	**203**	[177]	**204**	[179]	**205**	[181]	**206**	[181]	**207**	[175]	**177**	[81]
1	34.2	34.6	34.3	34.3	39.8	39.8	39.9	39.9	34.2	34.7	39.0	39.0
2	23.8	23.2	22.9	22.9	34.1	34.0	34.1	34.1	22.8	22.4	27.4	27.4
3	78.4	78.7	78.3	78.3	218	217	218	218	78.3	78.4	78.9	78.9
4	37.1	37.5	36.7	36.7	47.4	47.3	47.4	47.4	36.7	36.8	38.9	39.0
5	50.4	50.7	50.7	50.7	55.3	55.3	55.3	55.3	50.3	50.0	55.8	55.9
6	18.1	18.4	18.1	18.1	19.6	19.6	19.6	19.7	18.06	18.2	18.2	18.3
7	35.0	35.4	35.1	35.1	34.5	34.5	34.6	34.6	35.02	35.2	35.2	35.3
8	40.5	40.8	40.6	40.6	40.2	40.2	40.2	40.3	40.5	40.3	40.3	40.4
9	50.7	51.1	50.6	50.6	50.2	49.9	50.18	50.2	50.7	50.5	50.6	50.7
10	36.7	37.1	37.1	37.1	36.8	36.7	36.8	36.8	37.1	37.1	37.6	37.1
11	21.5	21.7	21.6	21.6	22.0	22.0	22.3	22.3	21.2	21.4	21.5	21.6
12	25.5	27.9	27.0	27.0	24.8	24.7	25.8	25.8	25.0	25.0	27.5	27.6
13	42.2	42.5	42.7	42.7	42.3	42.3	42.9	43.0	43.1	43.2	42.2	42.3
14	50.4	50.7	50.1	50.1	50.3	50.2	50.0	50.0	50.2	50.2	50.1	50.3
15	31.2	31.5	31.4	31.6	31.1	31.1	31.4	31.4	31.2	31.2	31.2	31.2
16	24.8	25.1	25.8	25.8	27.5	27.5	27.0	27.0	26.8	26.8	24.5	24.9
17	49.8	50.2	49.8	49.8	49.8	49.7	49.7	49.8	49.3	49.3	49.8	49.9

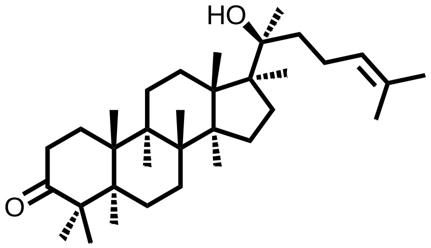
18	15.5	15.8	15.5	15.5	16.3	15.9	16.1	16.1	16.4	16.2	15.3	15.5
19	16.5	16.3	16.0	16.0	16.0	15.2	15.2	15.2	15.0	15.5	16.2	16.2
20	75.4	75.8	86.5	86.5	75.3	75.1	86.5	86.5	90.1	90.2	75.4	75.4
21	25.5	25.7	27.0	27.1	25.4	25.4	27.2	27.3	25.4	25.4	25.3	25.4
22	40.8	40.8	34.7	34.7	40.4	40.5	34.7	34.7	31.0	31.1	40.5	40.5
23	22.9	22.9	26.3	26.3	22.5	22.5	26.3	26.4	29.2	29.2	22.5	22.6
24	124.7	125.1	86.3	86.2	124.6	124.7	86.4	86.4	176.7	176.8	124.7	124.8
25	131.6	131.9	70.2	70.2	131.6	131.3	70.2	70.2	-	-	131.5	131.5
26	25.7	26.1	27.8	27.8	25.7	25.7	27.8	27.8	-	-	25.7	25.7
27	17.7	18.1	24.0	24.0	17.7	17.6	24.0	24.1	-	-	17.7	17.7
28	27.9	28.2	27.9	27.9	26.6	26.6	26.7	26.7	27.9	28.0	28.0	28.0
29	16.0	22.1	21.7	21.7	22.0	21.8	21.0	21.0	21.4	21.4	15.3	15.4
30	16.5	16.9	16.6	16.6	16.3	16.2	16.3	16.3	16.0	16.2	16.5	16.5
31	170.8	171.2	170.7	170.8	-	-	-	-	170.8	170.7	-	-
32	21.4	21.7	21.4	21.7	-	-	-	-	21.7	21.6	-	-

The signal due to C-20, C-21 and C-22 in the 13C NMR spectrum of **205** appeared at δ_C 75.3, 25.4 and 40.4, respectively. Table 19 shows a comparison of the C-13 chemical shift of C-20, C-21 and C-22 20-hydroxydammarenone II (20S) and 20-hydroxydammarenone (20S). Clearly the chemical shifts of three carbons in 20S closely agree with those reported for 20-hydroxydammarenone II confirming the stereochemistry of C-20 in **205** is S.

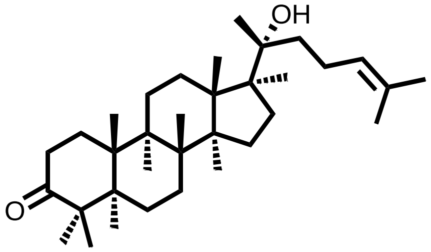
Hence, based on the spectroscopic data, **205** was identified as (20S)-20-hydroxydammar-24-en-3-one. Comparison of the NMR data with those reported in the literature for (20S)-20-hydroxydammar-24-en-3one revealed a very close resemblance as shown in Table 18 [176, 181]. Compound **205** was first isolated in 1955 by Mills from the resins of *Dipterocarpaceae* family, the so-called *Dammar* [182].

Table 19: spectral comparison of 20- hydroxydammarenone I and II with **205**

Compounds	C-20	C-21	C-22
20-hydroxydammarone II (20*S*)	75.1	25.4	40.5
20-hydroxydammarone I (20*R*)	75.6	23.5	41.8
Compound **205**	75.3	25.4	40.4



(20S)-20S-hydroxydammar-24-en-3-one
Hydroxydammarenone II



(20R)-20R-hydroxydammar-24-en-3-one
Hydroxydammarenone

Characterization of 20S, 24S-epoxydammaran-25-ol-3-one (206)

Cabraleone (**206**, BR5) was isolated as colorless oil. It showed a deep yellow spot at *Rf* = 0.50 (*ss1*) on TLC. Its UV spectrum gave an absorption maximum at 238 nm. It displayed IR absorption bands at 3528 cm^{-1} and 1688 cm^{-1} for hydroxyl and ketone functional groups, respectively.

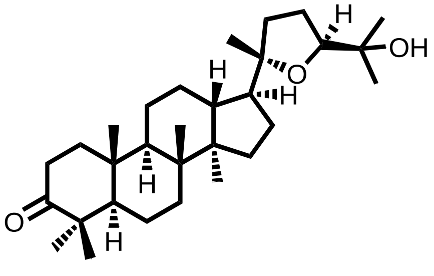
The ^{1}H NMR spectrum (Appendix 26) of **206** exhibited eight methyl proton signals at δ 0.88, 0.94, 1.01, 1.04, 1.08, 1.11, 1.15 and 1.19 all appeared as singlet. Multiplets appeared at δ 2.44 (2H, H-2), δ 2.48 (2H, H-1) due to methylene protons next to a carbonyl group. The doublet of doublets at δ 3.63 (*dd, J* = 9.8, 5.2 Hz) was allocated to the oxymethine proton at C-24. The deshielded methyl proton signals at δ 1.11, 1.15 and 1.19 can be attributed to the methyl groups attached to oxygenated carbons C-20 and C-25.

The ^{13}C NMR spectrum revealed 30 carbon signals as presented in Table 18 and Appendix 26. The DEPT-135 spectrum indicated the presence of eight methyl, ten methylene, five methine and seven quaternary carbons in the molecule. The most downfield signal at δ_C 218.1 is a characteristic signal for most triterpenes with a ketone function at C-3. The carbon signals at δ 70.2, 86.4 and 86.5 were suggestive of three oxymethine carbons designated for C-25, C-24, and C-20, respectively.

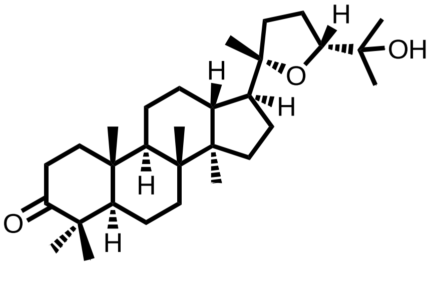
The stereochemistry of C-20 and C-24 were discussed the same way as compound **204** above. Comparison of chemical shifts of C-23, C-24, C-25 and H-24 in compound **206** were compared with the data reported for those ocotillone and cabraleone [183] as shown in Table 20. These two compounds they differ each other by the orientation of hydroxyl group at C-20. It was thus apparent that **206** had the same stereochemistry at C-20, C-23, C-24, C-25 and H-24 as cabraleone. Thus, **206** was identified as cabraleone based on the above spectroscopic data. Comparison of 1H and 13C data with those reported for 20S,24S-epoxydammar-25-ol-3-one [181, 182] revealed a very close agreement as shown in Table 19. **206** was first isolated from *Cabrdea* polyfricha [184].

Table 20: Spectral comparison of ocotillone and cabraleone with **206**

Compounds	C-23	C-24	C-25	H-24
Cabraleone (20S, 24S)	26.4	86.3	70.2	3.64 (*dd, J* = 10.0, 5.1Hz)
Ocotillone (20S, 24R)	27.4	83.3	71.4	3.73 (*dd, J* = 7.5, 7.5 Hz)
Compound **206**	26.3	86.4	70.2	3.64 (*dd, J* = 9.8, 5.2 Hz)



Cabraleone



Ocotillone

Characterization of Dammaranelactone-3α-acetate and dammar-24-endiol (207)

Dammaranelactone-3α-acetate (**207**, BR5) was isolated as colorless oil together with dammar-24-endiol (**177**) as an inseparable mixture with a ratio of 1:0.3. A characteristic pink spot was observed with *Rf* = 0.45 on TLC (*ss1*). The UV spectrum showed an absorption maximum at 241nm. The IR spectrum of the mixture showed two strong bands at 1763 cm^{-1} and 1726 cm^{-1} which were indicative of the presence of two ester functional group.

^{1}H NMR spectrum (Appendix 27) revealed the presence of seven singlet resonances at δ 0.84 (H-28), 0.87 (H-29), 0.89 (H-18), 0.94 (H-30), 0.97(H-19), 1.37 (H-21) and 2.09 (H-32) were assigned to methyl protons. Multiplet were observed at δ 2.66 (2H, H-2), δ 2.58 (2H, H-1) due to the methylene group at position adjacent to electron with drawing group. The most downfield broad triplet at δ 4.63 (*J* = 2.5 Hz) was suggestive of an oxymethine proton at C-3. The absence of additional oxymethine and olefinic proton resonance was evident from the 1H NMR spectrum. The fact that C-24 is a quaternary carbon atom and that a lactone ring may be present in the molecule could be inferred from the 1H NMR data in agreement with a previous report [175].

The ^{13}C NMR spectrum of **207** displayed the presence of 29 carbon signals as presented in in Table 18 and Appendix 27. With the help of the DEPT-135 spectrum, seven primary, ten secondary, five tertiary and seven quaternary carbons were identified. Signals appeared in carbonyl region at δ_C 170.8 and 176.7 which were indicative of the presence of an acetyl moiety and a lactone ring, respectively. The strong signals observed at δ_C 78.3 and 90.1 were suggestive of oxygenated carbons at C-3 and C-20, respectively. The NMR and IR spectroscopic data helped to identified **207** as dammaranelactone-3α-acetate. Comparison of the spectroscopic data with those for dammaranelactone-3α-acetate revealed a very close agreement as shown in Table 18 [181, 185].

All minor signals observed in the ^{1}H NMR spectrum of the mixture described above could be interpreted for **177**. The β-orientation of OH at C-3 was confirmed by the presence of *dd* signal at δ 3.2 (*J* = 11.1, 4.9 Hz, 1H) for H-3 [132]. The most downfield triplet at δ 5.1 (*J* = 7.2 Hz) was assigned to the olefinic proton at C-24.

The 30 minor signals in ^{13}C NMR spectrum of the mixture could be assigned to compound **177** as shown in Table 18. The two oxygenated carbon resonance at δ 78.9 and 75.4 could be attributed to C-3 and C-20, respectively. The olefinic carbon signals at δ 124.7 and 131.5 were indicative of the presence of a double bond at C-24.

Compound **177** was identified as (3β,20S)-dammar-24-endiol by comparing its ^{1}H and ^{13}C NMR data with those reported in the literature as shown in Table 19 [175]. (3β,20S)-dammar-24-endiol (**177**) was isolated for the first time from dammar resins of *Dipterocarpus* species [182]. Later, it was isolated from *B. frereana* in 1985 by Fatcorusso [81]. It is the first cyclization product of S-2,3-oxidosqualene [186]. Compounds **177** and **204** were isolated from the resin *Commiphora confusa* by Aman in 2003 [8]. To the best of our knowledge the aforementioned compounds have not been reported from the resin of *B. rivae*, however, **177** was reported from *B. frereana* [81]. Anti-tumor promoter [187], anti-inflammatory [188], cytotoxic [189] and anti-viral activities [190] of **177** and **204** have been reported.

2.3. Comparative studies of *Boswellia* species using qualitative TLC analysis

This section presents the comparative study conducted on the EtOH extracts of resins of *B. papyrifera* (BpR), *B. papyrifera* Kebtele (BpKR), *B. sacra* (BsaR), *B. serrata* (BsR), *B. frereana* (BfR), *B. pirottae* (BpiR), *B. neglecta* (BnR) and *B. rivae* (BrR), by TLC analysis. The TLC method demonstrated in this work provides a simple technique for the unambiguous chemotaxonomic identification of these eight species qualitatively.

The TLC chromatograms given in Figure 13 (A and B) were obtained by using solvent systems that achieved good separation of the neutral and acid fractions, whereas, the third chromatogram (C) shows the UV active spots in acid fraction at 254 nm. The first two chromatograms in Figure 13 (A and B) were developed by spraying with vanillin/H_2SO_4 followed by heating the TLC plates and then gradually cooling to room temperature. *Fig. 13A* and *13B* revealed the significant differences between the eight *Boswellia* species. Based on the chromatogram in *Fig. 13*, marker compounds in the resins were identified as discussed below (Table 21).

The marker compound specific to *B. papyrifera* Kebtele resin (BpKR) corresponding to light blue spot observed (at *Rf* = 0.39) and identified as cneoruben-10-ol (**119**). A marker compound is a compound found as a major or specific component in a given species. The second marker compound found in this resin gave a strong violet spot at *Rf* = 0.74 and was identifies as verticilla-4(20), 7, 11-triene (**104**). Thus far, compound 119 was only significantly detectable in BpKR, but not in other samples analyzed in this work. This finding is consistent with the report by Asmare [10].

Very specific for BpR was the existence of a yellow spot at *Rf* = 0.65 which was identified as incensole acetate (**111**). Furthermore, BpR showed consisted of a large amount of incensole (**109**) with a brown spot at *Rf* = 0.41 compared to *B. serrata* and *B. sacra*. Thus far, incensole acetate was only found the resin of *B. papyrifera*. This result is consistent with data published by Hamm *et al.* [6, 44], Camarda *et al.* [11] and Paul [48].

Hence, all publications by Moussaieff *et al*. [14], Banno [87], Akihisa *et al*. [67] and Basar [42] reporting the isolation of incensole acetate (**111**) from *B. sacra* (*Syn: B. carteri*) must be dealing with the resin of *B. papyrifera* instead.

B. papyrifera Kebtele (BpKR) was considered to be the same as *B. papyrifera* (BpR) but the chemical profile of the two resins are actually different. Compounds **111** and **109** were not detected in the extracts of BpKR. Verticilla-4(20),7,11-triene (**104**) is a major constituent of BpKR followed by cneoruben-10-ol (**119**). Compound **119** was totally not at all detected in the extract of BpR. In this work, spots on the TLC of BsaR and BcR were compared and found to be similar, thus confirming that *B. carteri* is the same as *B. sacra* [24]. The extract of BsaR showed a strong brown spot at *Rf* = 0.56, which corresponds to serratol (**108**). Likewise, the TLC of the extract of BsR showed strong spot at the *Rf* value identified as serratol (**108**). BsaR and BsR could be distinguished by the absence of pink spot at *Rf* 0.58 in BsR which is due to β-caryophyllene oxide (**98**) as mentioned by Paul [48, 149].

A very specific marker compound for BfR was observed as strong blue spot at *Rf* = 0.71 which was identified as α-phellandrene dimer (**118**). Other compounds were observed as deep violet spots at *Rf* = 0.70, 0.54 and 0.43, which were identified as, epilupeol acetate (**163**), epilupeol (**158**), and lupeol (**159**), respectively.

Yellow spots were observed at *Rf* = 0.54 in the TLC of the extracts of both BfR and BpiR. However, this spot was due to identified as different compounds in the two extracts. In the case of BfR, this yellow spot was identified as epilupeol, whereas, in the case of BpiR this spot corresponds to four compounds including epilupeol.

The yellow spots at *Rf* = 0.62 in the TLCs of the extracts of BpiR and BnR were found to be due to a mixture of compounds. In the extract BpiR the compounds were identified to be **129**, **148**, **157** and 3-oxo-tirucall-8,24-diene (**200**), whereas, in the extract of BnR **129**, **148** and **157** were detected. Furthermore, the common violet spot observed at *Rf* = 0.54 in both resins was identified as a mixture of **130**, **149** and **201** in BpiR, whereas, **158**, **130** and **149** were detected in BnR. The spot observed at Rf = 0.43 was identified as a mixture of **159, 131, 150** and **170** in BpiR, whereas **202** was detected instead of

170 in BnR together with **159, 131** and **150**. The presence of **200**, **201** and **170**, and the absence of **158** and **202** in BpiR distinguish this species from BnR.

The TLC chromatogram observed for BrR is different from BfR, BpiR, and BnR as shown in figure 13*.* In the neutral fraction of this resin five major spots were observed at *Rf* = 0.67, 0.61, 0.58, 0.50 and 0.45 which were identified as dammarenediol II acetate (**203**), cabraleone acetate (**207**), dammaren-20-ol-3-one (**205**), cabraleone (**206**) and cabraleone lactone acetate (**207**) together with 20S-dammar-24-ene-3β-diol (**117**), respectively. These dammarane-type triterpenes are marker compounds that differentiate this species from other *Boswellia* species analyzed in this work.

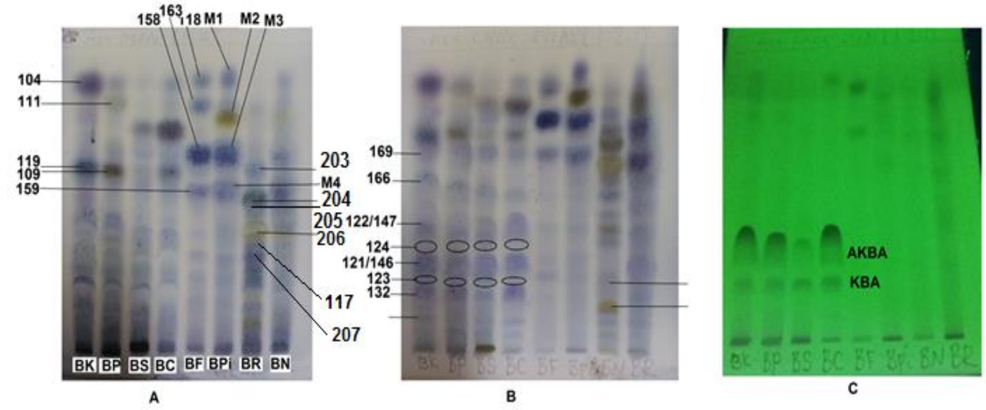


Figure 13: TLC chromatograms of resines:neutral fraction (A), acid fraction (B), after irradiation with UV lamp at 256 nm (C)

Solvent system: A: hexane:EtOAc (4:1) and B and C: hexane:CHCl3:EtOAc (1:2:1),
Mixtures: M1= **192-199**; M2 = **149, 148, 157, 200**; M3 = **130, 149, 201**; M4 = **131, 150, 159, 170**

As shown in *Fig. 13b* strong blue spots at *Rf* = 0.53 refer to 3-oxo-tirucall-8,24-dien-21-oic acid (**166**). When this spot was heated after visualizing, it first became a greenish and later turned blue and then gradually turned brown after 24 h. This is significant for compound **166** and thus useful for identification of BpKR, BpR, BsaR, and BsR from the rest of resins. However, this spot was not observed in the case of BfR, BpiR, BrR, and BnR. Moreover, a violet spot observed at *Rf* = 0.60 was indicative of 3α-acetyl tirucall-8,24-dien-21-oic acid (**169**).

As indicated in *Fig. 13b*, the polar parts of the extracts of the resins confirmed the presence of compounds with the same *Rf* value. The presence of boswellic acids in the resin of BpR, BpKR, BsaR, and BsR was confirmed by the dark spots visible under UV light at 254 nm. The spots at Rf = 0.25 and 0.36 (*Fig. 13C*) were identified as KBA (**123**) and AKBA (**124**), respectively. The absence of these dark spots can be taken as confirmation for the absence of the most potent boswellic acids (**123** and **124**) in the resin of BfR, BpiR, BnR, and BrR.

BpKR, BpR, BsaR, and BsR showed common spot at *Rf* = 0.43 (violet spot) identified as 3*α***-**acetyl-*α*-boswellic acid (**147**) and 3*α*-acetyl**-***ß*-boswellic acid (**122**). The violet spot at *Rf* = 0.30 was due to *α*-boswellic acid (**146**) and *ß*-boswellic acid (**121**). The spots at *Rf* = 0.36 and 0.25 were due to 3-*α*-acetyl-11-keto-*ß*-boswellic acid (**124**) and 11-keto-*ß*-boswellic acid (**123**), respectively. These compounds are typical markers for *Boswellia* species and can be monitored by detection using a 254 nm UV lamp. It was noticeable that extracts of BpKR and BpR showed the strongest AKBA spot. An article reported by Mannino *et al.* [191] in 2016 indicated that the amount of AKBA in BsaR (*syn.* BcR) was higher than BsR, whereas, KBA was higher in BsR. This fact was taken as a benchmark to compare the quantity of AKBA and KBA in BpR and BpKR in comparison with BsaR and BsR.

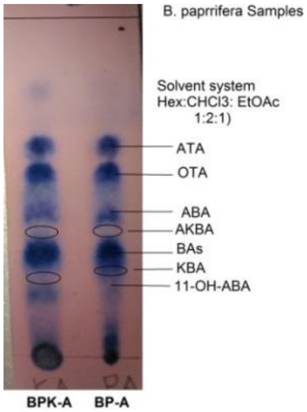


Figure 14: TLC chromatograms of acid fractions of *B. papyrifera* (K) and *B. papyrifera*

The neutral fractions of *B. papyrifera* (Kebtele) and *B. papyrifera* resins were compared as mentioned above. However, the acid fractions of *B. papyrifera* Kebtele resin (BpKR) and *B. papyrifera* resin (BpR) showed similar chemical profile on TLC as shown *Fig.* 14. The major spots observed were identified as 3-acetyl tirucallic acid (ATA), 3-oxo-tirucallic acid (OTA), a mixture of 3-acetyl α and β-boswellic acids, 3-acetyl-11-keto-β-

boswellic acid (AKBA), a mixture of α-and β-boswellic acids, 11-keto-β-boswellic acid (KBA) and 11-OH-β-boswellic acid.

Several sources of literature reported that *B. sacra* is synonyms with *B. carteri* [16-18]. In this work the resin of *B. sacra (Syn: B. carteri)* obtained from Yemen and Oman were compared with the resin *B. sacra* obtained from Somalia.

As shown in *Fig. 15*, TLC profiles of the resins of obtained from Somalia, Yemen and Oman were similar. The spot represented BS1, BS2, BS3, BS4 and BS5 were identified as mixtures of mono and diterpenes, serratol (**108**), 3-oxotirucallic acid (**166**), 3-acetyl tirucallic acid (**169**) and boswellic acids, respectively. However, the strengths of the spots differed, probably due to seasonal variations, geographical location and harvesting condition, among others. Serratol (**108**) and 3-oxo-tirucallic acid (**166**) are the two compounds that appeared as strong spots in the resin obtained from Somalia. The resin obtained from Yemen showed better serratol content than the resin from Oman. The major compound, serratol (**108**) in *B. sacra* distinguishes this species from other *Boswellia* resins.

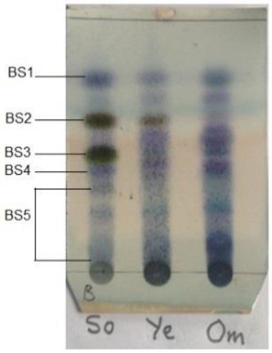


Figure 15: TLC chromatograms of extracts of the resins of *B. sacra* (So = SolamLia, Ye = Yemen and Om = Oman)

It is apparent from the TLC chromatogram of the B. sacra obtained from three sources (*Fig. 15*) had similar chemical profile. The differences between these resins could not be detected simply by observing spots of the polar region on TLC plate. For example, the spot represented by BS5 consisted of several triterpenes which had the same *Rf* value.

Table 21: TLC *Rf* values, solvent systems, colors of compounds identified from extracts of *Boswellia* resins

Compounds	*Rf*.	solvent	Color	Species	Remark
Verticilla-4(20),7,11-triene (**104**)	0.74	ss_1	Violet	BpKR, BpR	-
Cneoruben-10-ol (**119)**	0.39	‘’	blue	BpKR	-
Incensole acetate (**111**) n-Octyl acetate (**189)**	0.65	“	Yellow	BpKR	-
Incensole (**109)**	0.41	“	Brown	BpR, BsaR	-
Serratol (**108)**	0.56	“	Brown	BsaR BsR	-
Phellandrene dimmer (**118)**	0.71	“	Blue	BfR	-
ATA (**169**)	0.60	ss_2	Violet	BpR, BpKR, BsaR, BsR	-
3OTA (**166**)	0.53	‘’	Blue	“	-
ABAs (**122/147**)	0.43	“	Violet	“	-
AKBA (**124**)	0.36	“	-	“	Vis.
Bas (**121/146**)	0.30	“	Violet	“	-
KBA (**123**)	0.25	“	-	“	Vis.
Epilupeol acetate (**163**) α-Amyrin acetate (**191)**	0.70	ss_1	Violet	BfR	-
FAEs (**192-199)**	0.72	‘’	Violet	BpiR	-
α-Amyrone (**129)**, β-Amyrone (**148)** Lupenone (**157)**, Tirucallone (**200)**	0.62	“	Blue	BpiR, BnR	-
Epi-α-amyrin (**130)**, Epi-β-amyrin (**149**) Epilupeol (**158**), α-Tirucallol (**201**)	0.54	‘’	Violet	‘’	-
α-Amyrin (**131**), β-Amyrin (**150**) Lupeol (**159**), β-Tirucallol (**170**)	0.43	“	Violet	‘’	-
3β-OH-Tirucall-7,24-diene (**202**)	0.43	‘’	‘’	BnR	-
Dammarenediol II acetate (**203**)	0.67	‘’	violet	BrR	-
Cabraleone acetate (**204**)	0.61	‘’	green	“	-
Dammarenediol II (**205**)	0.58	‘’	violet	“	-
Cabraleone (**206**)	0.50	‘’	yellow	“	-
Cabraleone lactone acetate (**207**) Dammar-24-ene-3β-diol (**117**)	0.45	‘’	pink	“	-

Comparative analysis of the pyrolysates from *Boswellia* resins

In this section, the chemical profiles of the pyrolysate (incense or smoke) of *B. papyrifera* (Kebtele), *B. papyrifera*, *B. sacra* and *B. serrata* are compared with the EtOH extract of each resin, respectively. The resins were pyrolysed on locally made electrical stove and the smoke (incense) was trapped in CHCl3 as shown in *Fig. 17.*

Figure 16: Set-up of pyrolysis

Major diterpenes, **104** and **119** present in *B. papyrifera* Kebtele resin (BpKR), **109** and **111** in *B. papyrifera* (BpR), **98** and **108** in *B. sacra* (BsaR), and **108** in *B. serrata* (BsR) is also detected in the pyrolysate of the corresponding resins, BpKRs, BpRs, BsaRs, and BsRs, respectively. The presence of these compounds in each pyrolysate was confirmed by NMR by comparing the data with those generated for pure compounds isolated from extracts the resins (*Fig. 19*).

Visualization of the TLC chromatogram with UV lamp at 254 nm (*Fig.* 18) revealed the presence of additional identified spots (A-D) in the pyrolysates that were not detected in the solvent extracts of the resins suggesting chemical transformation upon heat treatment of the resins.

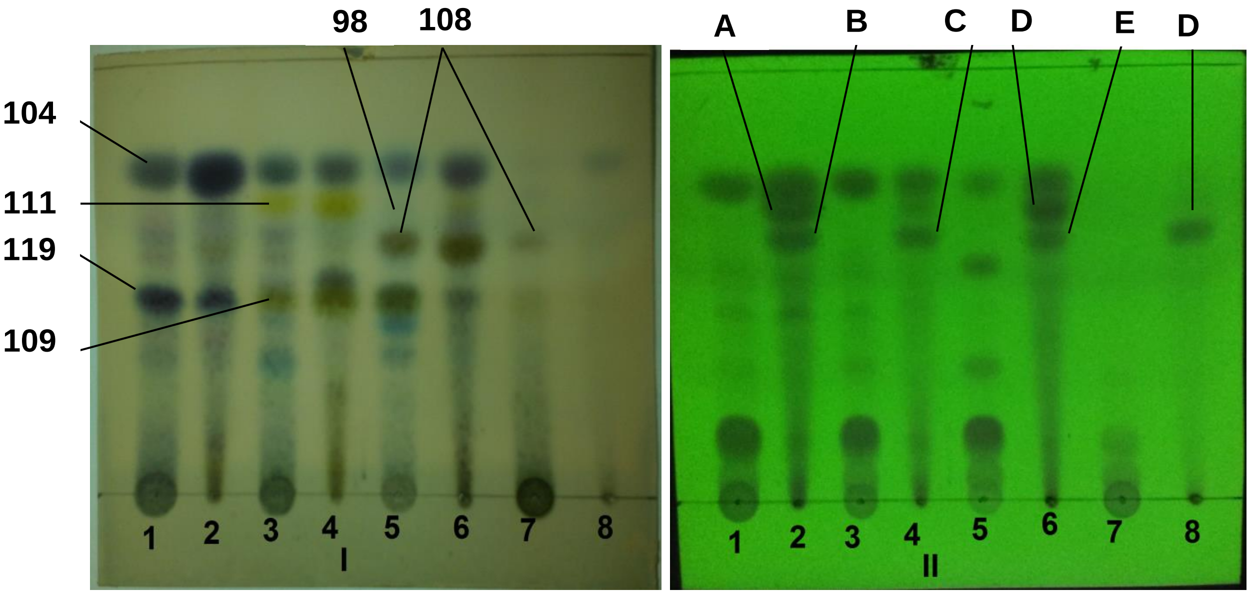


Figure 17: TLC chromatogram of pyrolysates of four *Boswellia* species:

1 (BpKR), 2 (BpKRs = *papyrifera* kebtele smoke),3 (BpR), 4 (BpRs = *papyrifera* smoke),

5 (BsaR), 6 (BsaRs = *sacra* smoke), 7 (BsR), 8 (BsRs = *serrata* smoke):

I: after spraying with vanillin/ H_2SO_4, II: under UV light at λ_{max} 254 nm.

To summarize this section, TLC is a useful technique to distinguish one species from the other qualitatively by observing the *Rf* value as well as the color of spots on the plate. The neutral fraction (hexane extract) of the resin is good enough to observe identifiable spots on TLC. However, to confirm the presence or absence of boswellic acids, EtOH extract is valuable. Four species, BpR, BpKR, BsaR (syn. BcR) and BsR, could be distinguished from other resins analyzed here by observing the presence of dark spot under UV light at 254 nm without spraying the TLC plate with a developing reagent. The dark spots were due to UV-active boswellic acids. In the remaining four resins, BfR, BpiR, BnR and BrR, these two UV-active boswellic acids were not detected. Undeniable specific marker compounds were incensole acetate (**111**) in *B. papyrifera*, cneoruben-10-ol (**119**) in *B. papyrifera* Kebtele resin, serratol (**108**) in *B. sacra and B. serrata*, α-phellandrene dimer (**118**) in *B. frereana* and dammarane derivatives in *B. rivae*. Whereas, the marker compounds in the remaining two species*, B. pirottae* and *B. neglecta,* were found as inseparable mixtures and were detected as a single spot on TLC.

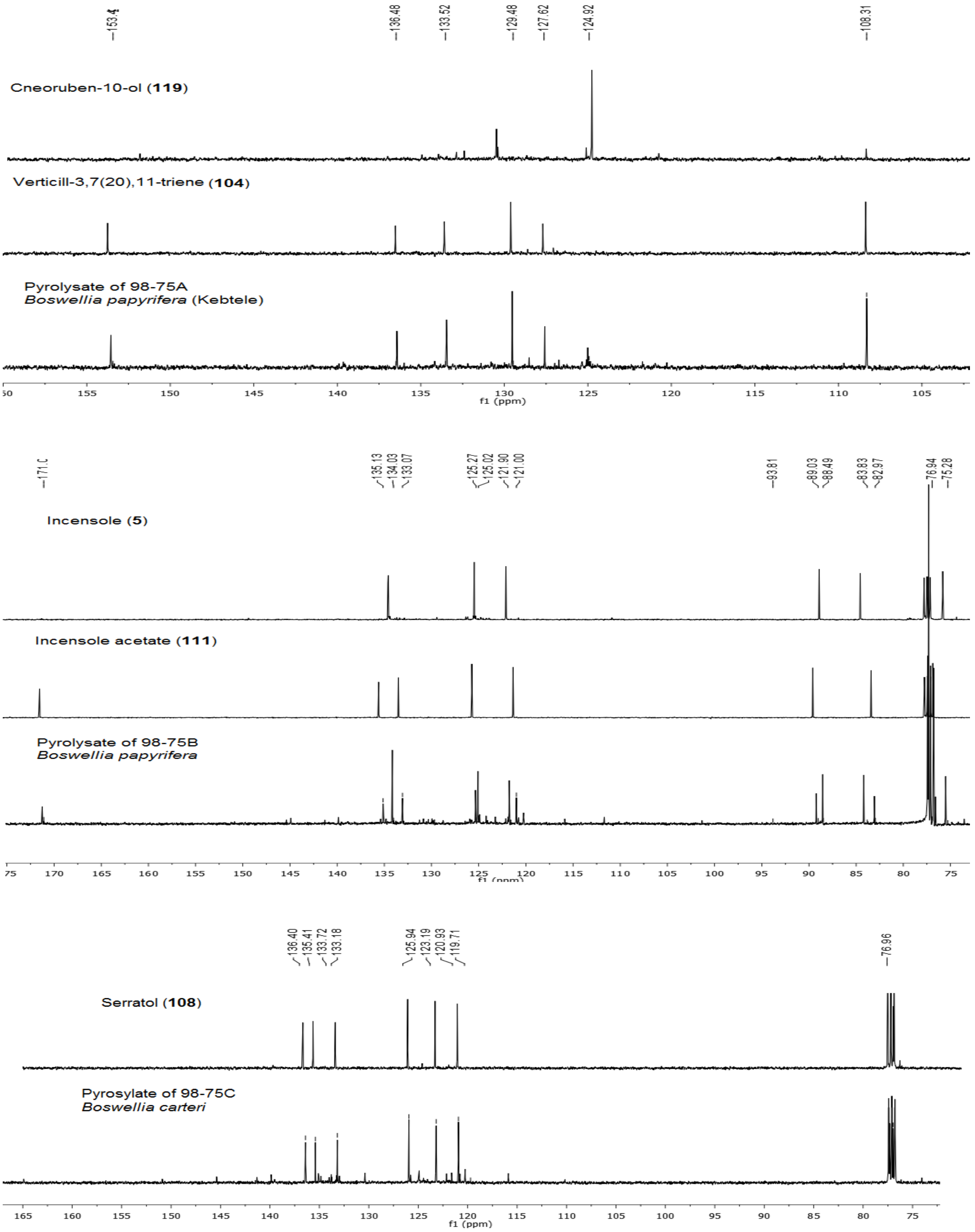


Figure 18: ^{13}C NMR data of pyrolysates of *Boswellia* species, cneorben-10-ol (**119**), verticill-3,7(20),11-triene (**104**), incensole (**5**), incensole (**111**) and serratol (**108**)

2.4. Analysis of major components of essential oils of *Boswellia* spp. by GC-MS

The essential oils of seven *Boswellia* species were obtained by steam distillation of the finely powdered oleogum resin. The yields of the volatile oils are presented in Table 22. The strongly aromatic oils were separated from the water layer by adding chloroform and dried with anhydrous Na_2SO_4. The oil obtained from *B. papyrifera* Kebtele resin was small when compared with the rest resins investigated here.

Table 22: Percentage of essential oils from *Boswellia* species

Boswellia species	Yield (g)	Oil content (%)
B. papyrifera resin	2.3	4.5
B. papyrifera Kebtele resin	1.0	2.0
B. sacra (Syn, B. carteri) resin	2.5	5.0
B. frereana resin	2.0	4.0
B. rivae resin	1.5	3.0
B. neglecta resin	2.0	4.0
B. pirottae resin	1.5	3.0

The essential oil were analyzed by GC-MS and components were identified through comparison of the fragmentation patterns in the resulting mass spectra with those published in the literature and by using National Institute of Standards and Technology (NIST) mass spectral database. Additionally, the data generated for the essential oils obtained from the resin of *B. papyrifera*, *B. neglecta* and *B. rivae* were compared with those reported by Aman [54], whereas, the data obtained from *B. sacra* and *B. frereana* data were compared with those reported by Hamma [46] and Mertens [15]. There have been no reports on the GC-MS analysis of the essential oils of *B. papyrifera* Kebtele resin. However, GC analysis of this resin was reported by Asmare with verticillantriene (**111**) as major and n-octyl acetate (**189**) as minor components [10].

As shown in Table 23 and *Fig. 20*, the major components of the essential oils of the *Boswellia* species analyzed in this work are n-octyl acetate (74%), verticillia-4(20),7,11-triene (76%), p-cymene (27%), terpene-4-ol (50%) and 3-carene (27%) in BpR, BpKR, BfR, BnR and BpiR, respectively. α-Pinene (48% and 36%) was the major constituent in BsaR and BrR. Other essential oils obtained from *B. papyrifera* Kebtele resin were cembrene A (**106**), cembrene C (**107**), verticillene (**208**), and isoverticillene (**209**). Some variations were observed with the data reported in the literature [15, 46, 54] and these may be due to seasonal and geographical conditions.

Table 23: Main components of essential oils from resins of *Boswellia* species

Rt	Major oils	BpKR	BpR	BsaR	BfR	BpiR	BnR	BrR
4.84	β-Thujene	-	-	-	12.0	6.5	2.6	3.6
4.94	α-pinene	-	-	48.0	6.1	20	9.0	37
5.07	Sabinyl propipnoate	-	-	-	2.1	-	-	-
5.44	β-Phellandrene	-	-	14.0	3.2	7.0	0.8	-
5.50	β-pinene	-	-	-	-	-	-	6.0
5.61	β-myrcene	-	-	10.5	-	-	-	-
5.82	α-Phellandrene	-	-	-	-	-	-	4.0
5.85	Sabinene	-	-	-	-	7.3	-	-
5.89	3-Carene	-	-	9.0	-	22	-	-
6.06	p-Cymene	-	-	-	27.0	-	6.7	23
6.17	E. β-Ociemene	-	-	10.6	-	-	-	-
6.11	Limonene	-	-	-	-	1.1	6.1	18
6.31	o-cymene	-	-	-	-	9.0	-	-
6.46	γ-Tepinene	-	-	-	-	2.0	1.0	-
6.53	n- Octanol	-	8.0	-	-	-	-	-
7.15	Linalool	-	-	-	3.0	-	-	-
7.42	α-Thujen-ol	-	-	-	-	-	-	3.0
7.46	Trans- Verbenol	-	-	-	-	8.0	3.7	5.9
7.82	Terpene-4-ol	-	-	-	8.0	1.5	51	-
7.95	α-Terpineol	-	-	-	2.0	-	16.0	-
7.96	α-Phellandren-8-ol	-	-	-	-	1.2	-	-
8.06	n-Octyl acetate	2.7	74.2	-	2.5	-	-	-
8.18	Cis-verbenone	-	-	-	-	-	2.0	-
9.92	β-Bourbonene	-	-		3.0	-	-	-
11.70	Carophyllene	-	-	7.6	-	-	-	-
15.80	Cembrene	-	4.0	-	-	-	-	-
16.60	Dimer of α-phellandrene	-	-	-	12.2	-	-	-
19.30	Incensole acetate	-	6.5	-	-	-	-	-
19.80	Cembrene C	2.4	-	-	-	-	-	-
20.09	Isoverticillene	1.1	-	-	-	-	-	-
20.49	Verticillatriene	76.3	7.2	-	-	-	-	-
20.60	Verticillene	5.3	-	-	-	-	-	-
22.87	Cneorben-10-ol	7.2	-	-	-	-	-	-
	Total	95.0	99.9	99.7	81.0	84.3	99.0	99.6

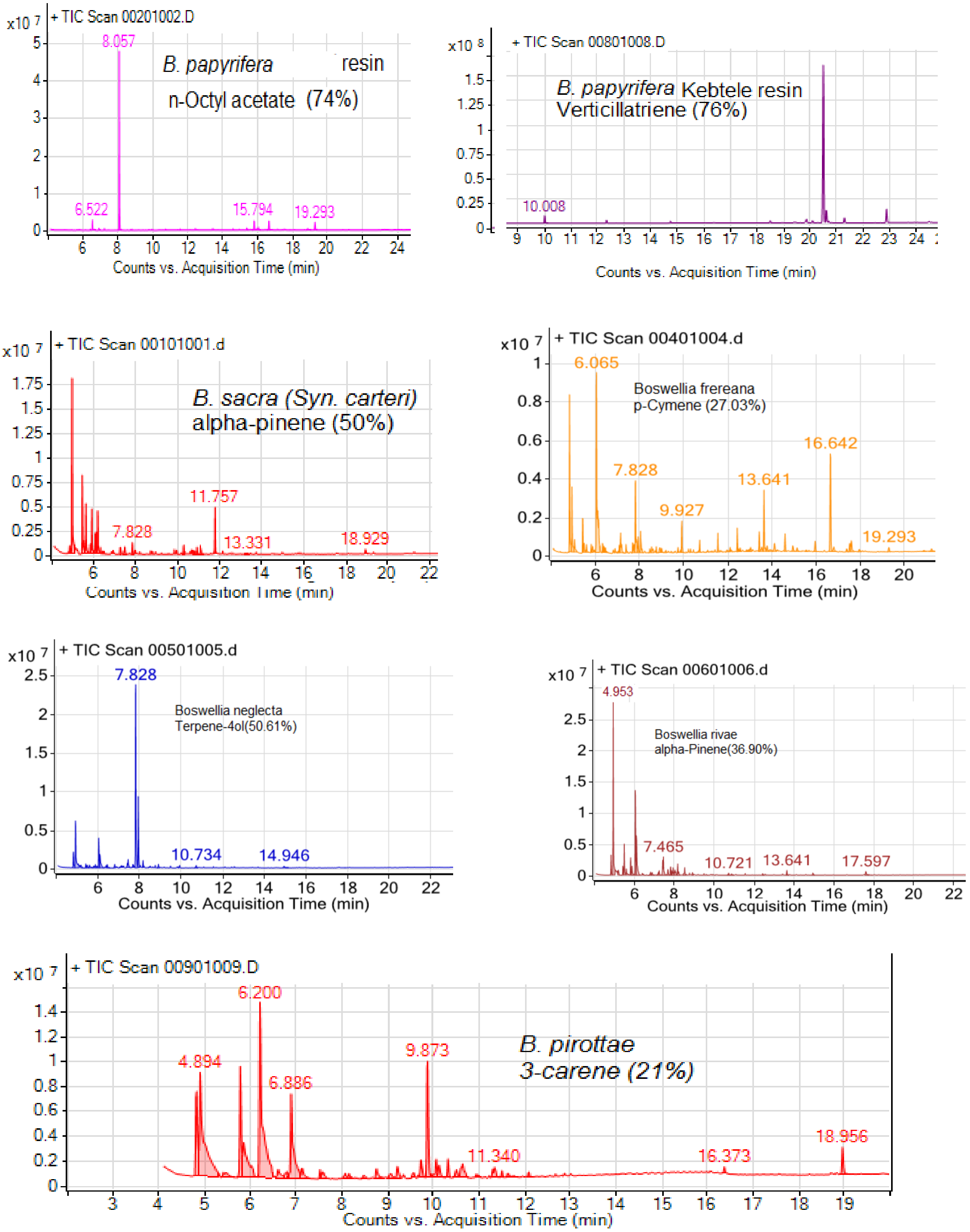


Figure 19: GC-MS Chromatograms of the oils of *Boswellia* resins

2.5. Quantification of AKBA and KBA in resin of *Boswellia* species by HPTLC

The CAMAG HPTLC system equipped with a sample applicator Linomat V, TLC Scanner IV, Twin trough glass Chamber and Wincats an integrated Software 4.02 was used for quantification of compounds in the EtOH extracts. For the quantitative analysis of *Boswellia* species towards the potent anti-inflammatory compounds, 3α-acetyl-11-keto-β-boswellic acid (AKBA, **124**) and 11-keto-β-boswellic acid (KBA, **123**), only four species were selected, i.e., BpKR, BpR, BsaR, and BsR, based the results obtained from qualitative analysis of the resins. Standards and samples were prepared as 1 mg/mL and 5 mg/mL solutions, respectively, and kept in a freezer until analysis.

The TLC procedure was optimized with a view to quantifying AKBA and KBA in four *Boswellia* resin extracts. The mobile phase consisting of ss_2 gave a good resolution and a sharp and well-defined peak at $R_f = 0.36$ for AKBA and 0.25 for KBA in the standard as well as in the extracts. Well-defined spots were obtained when the developing chamber was saturated for 10 min at room temperature that enabled to accurately quantify AKBA and KBA in the diluted solutions.

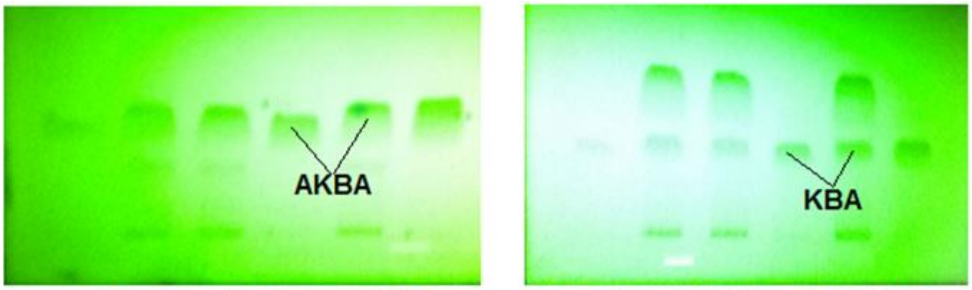
To prepare the calibration curve, 1.0, 2.0, 3.0, 4.0 and 5.0 μl of the standard solution were spotted. Tracks 1, 3 and 5 were considered as standards and the remaining tracks 2 and 4 were considered as unknown samples. The TLC method developed for estimation of AKBA and KBA showed a good correlation coefficient ($r_{AKBA} = 0.99634$, $r_{KBA} = 0.98179$) in the concentration range of 1 to 5 μg spot^{-1} with respect to the peak area scanned at 254 nm. The amount of AKBA on Track 2 and 4 were quantified as 2.2 and 4.0 μg, whereas, the amount of KBA were 1.7 and 3.8, respectively.

Table 24. Repeatability of the method (in μg)

Sample	Wt. (μg)	Replicates (μg)			mean	S.D	R. S .D	%R.S.D
		1	2	3				
AKBA	4.0	4.080	3.667	4.001	3.916	0.2192	0.0559	5.5
KBA	4.0	3.789	3.954	4.023	3.922	0.1202	0.0306	3.0

Precision, repeatability, of the sample application and measurement of peak areas were carried out using three replicates of the same spots (4.0 μg) of AKBA and KBA. The percent relative deviation (% R.S.D) for repeatability of sample application was found as 5.5% and 3.0% for AKBA and KBA, respectively, as shown in Table 24.

The accuracy of the quantification was assessed in a recovery study (Table 25). One of the samples analyzed here was spiked with extra 0.5, 1.0 and 1.5 μg of both standards AKBA and KBA separately and reanalyzed as shown in *Fig.* 21. The proposed method, when used for estimation of AKBA, afforded recovery of 94.8 to 100%, and for estimation of KBA 94.2 to 98.8%, which were acceptable values.



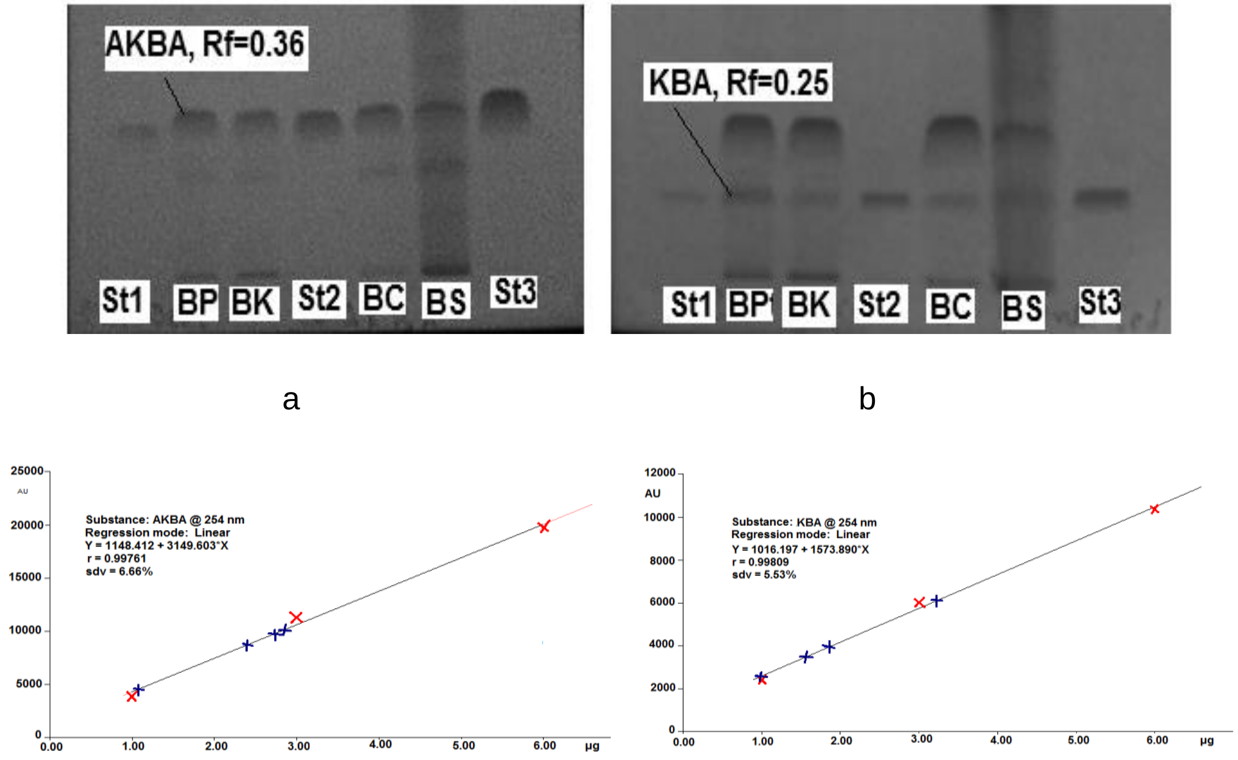
a: AKBA spiked Bpap(T) EtOH **b: KBA spiked Bpap(T) EtOH**

Figure 20: TLC chromatogram of spiked Bpap samples (a: AKBA and b: KBA)

Table 25. Results of recovery study

	Amount sample (µg)	Spiked amount (µg)	Theoretical value (µg)	Experimental mean value (µg)	Recovery (%)
AKBA	2.8 µg	0.5 µg	3.3 µg	3.13 µg	94.8
	2.8 µg	1.0 µg	3.8 µg	3.79 µg	99.7
	2.8 µg	1.5 µg	4.3 µg	4.31 µg	100.3
KBA	1.6 µg	0.5 µg	2.1 µg	1.98 µg	94.2
	1.6 µg	1.0 µg	2.6 µg	2.57 µg	98.8
	1.6 µg	1.5 µg	3.1 µg	3.05 µg	98.4

The EtOH extracts of BpKR, BpR, BsaR and BsR were analyzed three times using HPTLC and the results are summarized in Table 26. Single spot at R_f = 0.25 and 0.36 were observed in the chromatograms of KBA and AKBA. There was no interference in the analysis from other components present in the extracts. The curves for the estimation of AKBA and KBA are shown in *Fig.* 22. The three-dimensional spectra and spectral comparison of in four samples mentioned above are also indicated in *Fig.* 23.



a b

c d

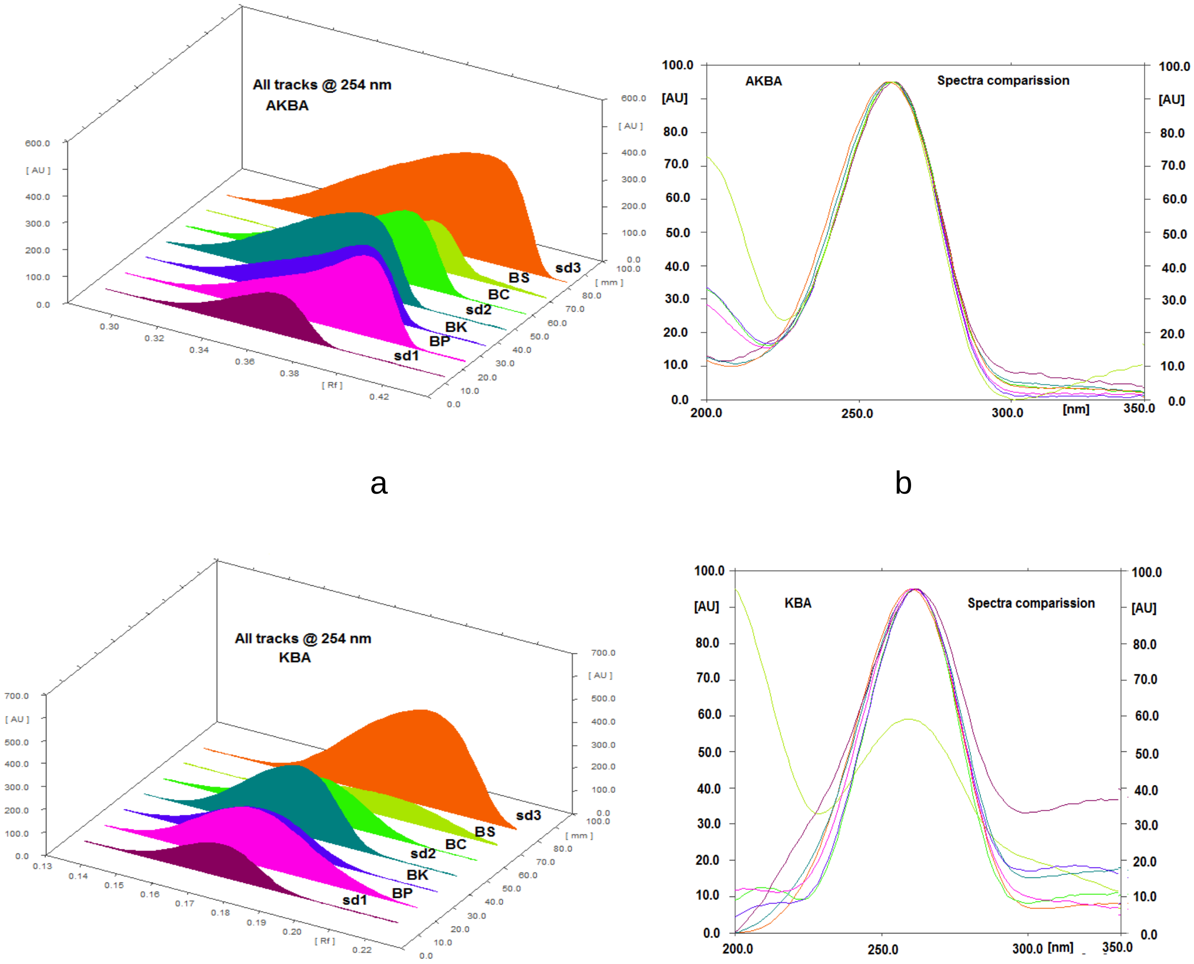
Figure 21: TLC chromatogram and a calibration curve of AKBA (a, c) and KBA (b, d)

Table 26: Summary of quantitative TLC results

Resin Sample	applied volume	amount applied spot^{-1}	amount in extract KBA spot^{-1}	% KBA	AKBA spot^{-1}	% AKBA
BpR	4 µL	20 µg	1.6 µg	8.0	2.8 µg	14.0
BpKR	4 µL	20 µg	0.8 µg	4.0	2.4 µg	12.0
BsaR	4 µL	20 µg	1.0 µg	5.0	2.7 µg	13.5
BsR	4 µL	20 µg	0.7 µg	3.5	1.4 µg	7.0

The amount of AKBA found in 20 µg of EtOH extract of BpR, BpKR, BsaR, and BsR was 2.8 µg (14.0%), 2.4 µg (12.0%), 2.7 µg (13.5%), and 1.4 µg (7.0%), respectively.

Whereas, the amount of KBA quantified was 1.6 µg (8.0%), 0.8 µg (4.0%), 1.0 µg (5.0%) and 0.7 µg (3.5%), respectively. The amounts of the most potent anti-inflammatory and anti-cancer compounds found in the resin of *B. papyrifera* were higher than those found in the well-known Indian species *B. serrata* and Somalian species *B. sacra* [191].



a

b

c

d

Figure 22: Three-dimensional spectral and spectra comparison of AKBA (a, b) KBA(c, d)

3. Conclusions

The main aim of this study has been the chemical investigations of two *Boswellia* species, i.e., *B. papyrifera* and *B. pirottae*. *B. pirottae* is a hitherto uninvestigated rare and endemic species to Ethiopia. From the ethanol extract of the resin of *B. pirottae*, a total of 19 compounds, i.e., 11 triterpenes, and 8 fatty acid esters, were isolated and identified. To the best of our knowledge, this is the first report on the isolation and identification of the aforementioned compounds from *B. pirottae*.

The *Boswellia* species found in the Amhara region (Agew-Awi zone, Jawe woreda, particularly Kebtele kebele) was found to be different from *B. papyrifera* which occurs in the Northern parts of Ethiopia because of the unusual occurrence of diterpene cneorubenol (**119**) as a marker compound. A total twelve known compounds, i.e., two diterpenes, one fatty acid, two tirucallic acids and seven boswellic acids, were isolated from *B. papyrifera* Kebtele resin, of which ten compounds are reported in this work for the first time from this resin. The GS-MS analysis of the oil of *B. papyrifera* Kebtele resin showed that verticillan-4(20),7,12-triene (**104**) and cneorben-10-ol (**119**) are the major components of the oil, whereas, n-octyl acetate (**189**), and incensole acetate (**111**) are major components of the oil of *B. papyrifera* resin.

Comparative studies of eight resins from *B. papyrifera*, *B. papyrifera* Kebtele collection, *B. sacra, B. serrata, B. frereana, B. pirottae*, *B. neglecta* and *B. rivae* by TLC and GC-MS helped us to differentiate one resin from the other by means of marker compounds. Among the *Boswellia* species analyzed in this work, the typical marker compounds found in five resins are incensole acetate (**111**) in *B. papyrifera*, cneoruben-10-ol (**119**) in *B. papyrifera* Kebtele resin, serratol (**108**) in *B. sacra and B. serrata*, α-phellandrene dimer (**118**) in *B. frereana* and dammarane derivatives in *B. rivae*. The marker compounds in the remaining two species*, B. pirottae* and *B. neglecta,* were found as an inseparable mixture at a given *Rf* value.

The resin of *B. papyrifera* is identifiable by TLC and GC-MS techniques. Previously, this species was often confused in the literature [24, 149] with *B. sacra*, though there have been sufficient publications which arrived at similar conclusions as stated in the work presented here.

The GC-MS analysis of the essential oils of the resins can be used to differentiate one resin from the other as shown in this work. The major components of the essential oils were found to be n-octyl acetate, verticilla-4(20),7,11-triene, p-cymene, terpene-4-ol and 3-carene in *B. papyrifera*, *B. papyrifera* Kebtele resin, *B. frereana*, *B. neglecta* and *B. pirottae*, respectively. In the oils obtained from the resins of *B. sacra* and *B. rivae,* α-pinene was found to be the major constituent.

In this work, the amounts of two the most active ingredients, AKBA and KBA, were detected in four resins by HPTLC using a CAMAG TLC scanner IV. The results indicated that the amounts of two compounds found in *B. papyrifera* were higher than in the other resins analyzed here. Accordingly, the amounts of AKBA and KBA found in the EtOH extract of *Boswellia papyrifera* were quantified as 14 and 8%, respectively.

Frankincense resins are usually used as fumigants in cultural and religious ceremonies. We therefore analyzed the smokes of resins of *B. papyrifera*, *B. sacra* and *B. serrata* obtained by gentle burning and then capturing smoke constituents by extraction using solvents. Most of the compounds present in the resin were found in the smoke.

Finally, we believe this study has made modest contribution to show that this unique Ethiopian frankincense compares favorably with the better known resins such as Indian *B. serrata* and Oman *B. sacra*.

4. Experimental

4.1. Resin materials, solvents and chemicals

Most of the resin materials used for the investigations reported here were obtained from the natural product research lab of AAU department of chemistry. The material was already correctly assigned as authentic frankincense resin material in the stock of professor Ermias's research lab collection and also fresh samples were properly identified by National Herbarium, AAU.

Table 27: Lists of Resins Investigated

Scientific name	Abrv.	Local name	Book code	Herbarium number	Collection
B. papyrifera	BpR	*Nech etan* (Amh.)	98-8A	sisay-01-2016	Sekota
*B. papyrifera (*Kebtele*)*	BpKR	*Walya meker* (Amh)	98-34G	A. Melese	Agew-Awi
B. sacra (Syn. B. carteri)	BsaR	*Beyo*	98-37E	E. Dagne	Somalia, Oman
B. serrata	BsR	*salai guggal*	98-8E	‘’	India
B. frereana	BfR	*Maydi*	98-39A	‘’	Somalia
B. pirottae	BpiR	*Tantani* (Orom.) *Yetan-Zaf* (Amh)	98-46A	072815 (sisay-01-2017) 072817 (sisay-02-2017)	Gibe valley Chis-Abay
B. neglecta	BnR	*Borena Etan* (Amh.)	98-9B	072813	Borena
B. rivae	BrR	*Ogaden Etan* (Amh)	98-9C	072808	Ogaden

All chemicals and solvents used for quantification, GC-MS analysis and UV-analysis were analytical reagent grade unless stated otherwise. NMR spectra were normally recorded in deuterated chloroform ($CDCl_3$, 99.8 % D) if not stated otherwise. AKBA and KBA used as a standard for HPTLC quantitation were isolated from *B. papyrifera* resins by column chromatography and purified by PTLC. Any peculiarities and differences from the descriptions given here are mentioned in the corresponding experimental chapters.

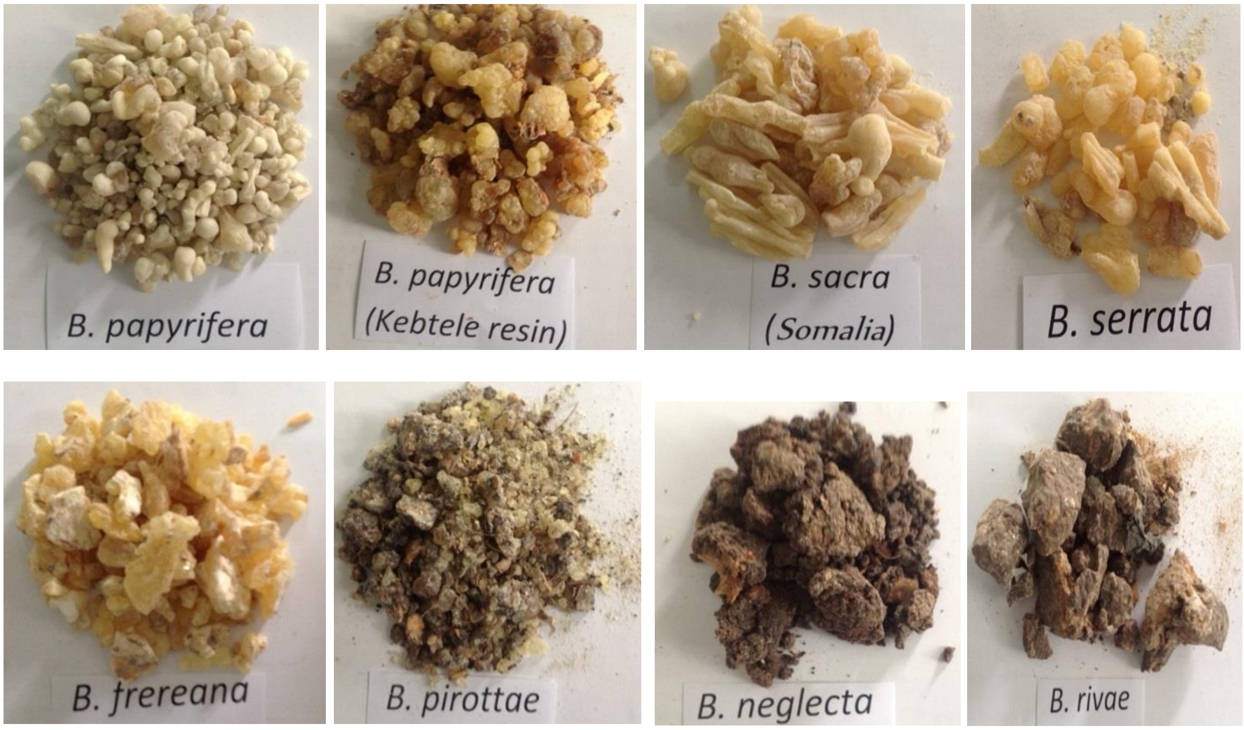


Figure 23: Photos of resins (By Professor Ermias Dagne)

4.2. Extraction procedure

4.2.1. Selection of extraction solvents

The suitable solvent for extraction of the resins was determined by stirring 5 g powdered resin in 10 mL solvent using magnetic stirrer for 30 min. The percentage of dissolved sample was calculated by dividing the mass of crude extract of samples by 5.

Table 28: Amount of resins extracted (%)

Boswellia species	Short (Book) code	Hexane	$CHCl_3$	EtOH	Water
B. papyrifera	BpR (98-8A)	24	48	80	18
*B. papyrifera (*Kebtele resin)	BpKR (98-34G)	21	46	80	14
B. sacra (Syn. B. carteri)	BsaR (98-27F)	50	65	78	18
B. serrata	BsR (98-8E)	22	51	75	16
B. frereana	BfR (98-39A)	90	77	79	0.5
B. pirottae	BpiR (98-46B)	85	74	65	9.0
B. rivae	BrR (98-8C)	80	57	60	30
B. neglecta	BnR (98-8B)	74	55	65	22

4.2.2. Extraction of resins for qualitative and quantitative analysis

20 g of coarsely powdered resin samples were stirred in 60 mL EtOH using magnetic stirrer for 4 h. The extracts were filtered by Whatmann filter paper and concentrated and kept in proper place for quantification of boswellic acids 50 mg crude extract of each sample was dissolved in $CHCl_3$ up the mark in 10 mL volumetric flask to make 5 mg/mL stock solution for quantification of AKBA and KBA.

4.2.3. Acid-base extraction of samples for isolation of pure compounds

Neutral and acidic fraction were obtained as follow [43, 58], 20 g of powdered frankincense gum-resins were dissolved in 60 mL of ethanol by stirring using magnetic stirrer for 4 hs. The insoluble ingredients of the resin were discarded by filtration and the solutions were condensed under vacuum to a viscous liquid to a volume of 30 mL. The resulting viscous liquid was treated with 3% KOH solution (pH=10). The neutral phase was separated using ethyl acetate to afford nonpolar fraction which is known as a neutral fraction in this Thesis unless and otherwise specified (*Eg.* BpRn = *Boswellia papyrifera* resin neutral fraction). The neutral fraction was concentrated and kept for isolation of marker compounds. The aqueous extracts were combined and acidified with 2% HCl (pH=3). The organic fraction was separated from aqueous using ethyl acetate. The final ethyl acetate phase was evaporated to dryness affording the acidic fraction of the resin (*Eg*. BpRa = *Boswellia papyrifera* resin acid fraction) which named as aid fraction in this Thesis.

The coding system indicated that, for example BPK4, B stands for the genus name *Boswellia*, P stands for the species of resin, K stands for Kebtele (place) and number 4 behind K stands for the location of the spots of compounds on the TLC plate starting from the highest *Rf* value to the lowest after sprayed with vanillin/H_2SO_4 reagent.

4.2.4. Hydro-distillation of resin samples for GC-MS analysis

Finely ground oleogum resins (50 g of each) were subjected to hydro-distillation using Clevenger's apparatus until complete exhaustion. The oils were collected, dried over anhydrous sodium sulfate and kept in the refrigerator until analysis. The data was recorded using GC-MS Agilent Technologies. GC-system-7820A, MS- 5977E MSD detector with column size 30m x 0.250mm, 0.25 micron- 60 to 350 ^{o}C.

4.3. Thin-layer and column chromatographic technique

4.3.1. Thin-layer- chromatography

TLC Silica gel 60 F_{254} Multi-format pre-scored to 20 x 20 cm aluminum coated plates (stationary phase) were used. Two mobile phases standardly consisted of a mixture of hexane and EtOAc (4:1), known as solvent system 1 (ss_1), for neutral fraction of the resins, and hexane, chloroform and EtOAc (1:2:1), known as solvent system 2 (ss_2), for the acid fraction of resins were used. TLC plate start lines were drawn (pencil) at 1.5 cm; giving an elution distance of maximal 4 cm. Spots were put on with a small capillary. UV detection (254 nm and 360 nm) was carried out by UV light source. Chromatograms were further developed by vanillin/H_2SO_4 reagent. After the development of the chromatograms, the TLC plate was completely sprayed by a solution of this dyeing reagent and heated with a heat plate at 110 ^{o}C until the colored spots appeared. The spots were visually detectable after color development at room temperature.

4.3.2. Column chromatography

Normal phase silica gel (stationary phase) with particle size 70-230 µm was used. The mobile phase standardly used was a mixture of hexane, chloroform, ethyl acetate and methanol with increasing polarity. MeOH was used as eluent when the acid fraction of resins was applied on a column. A step gradient, each step 100 mL, of increasing polarity for raw material (neutral and acid fraction) separations was applied. Silica gel was suspended in the hexane, shortly degassed in a vacuum and filled into the column. The sample was evenly added on top of the column bed after the sample was adsorbed inside the silica gel. Fractions were collected using 50 mL collecting vials in a volume of 30 mL. Fractions were monitored by TLC and combined with overall fractions due to their chromatographic analogies. The chromatography itself followed basically the instructions of Still *et. al* [192].

4.3.3. HPTLC equipment and quantification method

A CAMAG (Switzerland) HPTLC system equipped with a sample applicator Linomat V, Twin trough glass Chamber (20x10 cm^2) with SS lid, TLC Scanner IV and Wincats an integrated Software 4.02 (Switzerland). HPTLC Glass pre-coated plate with Silica gel F254 (20x10 cm^2; 0.2 mm thick) was used with optimized mobile phase ss$_2$ gave a good resolution, and a sharp and well-defined peak at R_f = 0.36 for AKBA and 0.25 for KBA in the standard as well as in the extracts. Well-defined spots were obtained when the developing chamber was saturated for 10 min at room temperature.

EtOH extract of samples and standard solutions (AKBA and KBA) were applied on a plate by using Linomat V applicator. HPTLC plate was developed to 8 cm distance above the position of the sample application. The plate was removed from the chamber and air dried at room temperature. AKBA and KBA are UV active compounds that make the quantitative analysis easy. HPTLC fingerprint profile was snapped by Huawei G730-U10 Mobile Camera under UV 254 nm. For the quantification of AKBA and KBA the TLC plate was scanned immediately using CAMAG TLC Scanner IV at wavelength 254 nm without derivation.

In order to establish linearity, standard solutions of AKBA and KBA (1mg/mL) were applied to TLC plate, 1.0 µL, 3.0 µL, 6.0 µL on track 1, 4 and 7, respectively, and for AKBA assay, 4.0 µL of EtOH extract of BpR, BpKR, BsaR, and BsR were applied on track 2, 3, 5 and 6, respectively. However, 8 µL of each sample was applied on respective tracks for the quantification of KBA. Wincats an integrated Software 4.02 was used for the detection as well as for the evaluation of data from peak area and height [193-195].

4.3.4. Spectroscopy

The measurements of ^{1}H, ^{13}C, DEPT, HSQC, HMBC- spectra were performed with instruments Bruker ACQ 400 AVANCE spectrometer operating at 400 MHz. The IR spectra were recorded using a Perkin-Elmer BX Spectrometer (400-4000 cm^{-1}) in KBr. UV data were recorded using T60 UV-VS spectrophotometer.

4.4. Fractionation and isolation of compounds from *Boswellia* resins

4.4.1. Isolation of compounds from *B. papyrifera* (Kebtele collection, BpKR)

A neutral fraction of *B. papyrifera* Kebtele resin (2 g) (Section 4.2.3) was adsorbed in silica gel with methanol and subjected on normal phase column packed with silica gel (70 g, particle size 70-230 µm). Elusion has carried out with a gradient of solvents Hexane, $CHCl_3$ and EtOAc ratio with increasing polarity of the eluent to afford 13 fractions. Fraction 1 and Fraction 3 contain one major spot each, Fraction 2 contains two major spots observed in Fraction 1 and Fraction 3, and Fractions 4-13 contain spots observed in the acid fraction of the resin. Fraction 1 and Fraction 3 were applied on a small column packed with silica gel (17 g) to furnish verticilla-4(20),7,11-triene (**104,** BPK1**,** 150 mg) and cneorubenol (**119,** BPK5**,** 100 mg), respectively.

The acid fraction of this resin (2 g) was applied on a column packed with silica gel (70 g, particle size 70-230 µm). Elusion was carried out with the same procedure as a neutral fraction to afford 10 fractions. Fraction 1 (200 mg) was applied on a column and repeated purification afforded three compounds: fatty acid (**186**, BPK6, 5 mg), 3α-acetyl-tirucall-8,24-din-21-oic acid (ATA, **169**, BPK7, 15 mg) and 3-oxo-tirucall-8,24-dien-21-oic acid (3-OTA, **166**, BPK8, 20 mg). Fraction 2 (200 mg) was applied on a small column to isolate a mixture of 3α-acetyl-α-boswellic acid (AαBA, **147**, BPK9) and 3α-acetyl-β-boswellic acid (AβBA, **122**, BPK10). Fraction 3 (500 mg) was applied on a column followed purification using PTLC to obtained AKBA (**124**, BPK11, 15 mg), Fraction 4 (200 mg) was applied on a column to obtain a mixture of α-BA (**146**, BPK13) and β-BA (**121**, BPK12). Fraction 5 (150 mg) was fractionated in the same way to afford 11-OH-BA (**132**, BPK14, 5 mg) and KBA (**123**, BPK15, 5 mg).

Verticilla-4(20), 7, 11-triene: Colorless viscous oil (100 mg); soluble in $CHCl_{3;}$ Rf = 0.74 (ss_1, vanillin/H_2SO_4); IR $^{\upsilon}{}_{cm-1}$(KBr): C=C (1646), C-H (2923_s, 1441_b); ^{1}H NMR (400 MHz, CDCl3), chemical shift δ in ppm, coupling constant *J* in Hz: δ_H 1.46-1.53 (1H, *m*, H-1), 1.64-1.62 (2H, *m*, H-2), 2.78 (2H, *m*, H-3), 2.04-2.07, 2.26-2.30(2H, *m*, H-5), 2.16-2.23 (2H, m, H-6), 5.15 (1H, *dd*, *J* = 9.2, 3.6 Hz, H-7), 2.47(2H, *td*, *J* = 8.2 and 4.4 Hz, H-9), 2.03-2.07, 1.85-1.89 (2H, m, H-13), 2.1-2.16, 2.20-2.30 (2H, *m*, H-14), 1.01 (3H, *s*, H-16), 0.99 (3H, *s*, H-17), 1. 71 (3H, *s*, H-18), 1.64 (3H, *s*, H-19), 4.70, 4.64 (2H, s, H-20).

Cneoruben-10-ol: Yellowish viscous oil (75 mg); Mw: $C_{20}H_{34}O$; soluble in $CHCl_{3;}$ *Rf* = 0.68 (ss_1, vanillin/H_2SO_4); IR υ_{cm-1}(KBr): 3429 (O-H), 1656 (C=C), 1451 (C-H), 1373 (C-O); ^{1}H NMR (400 MHz, CDCl3), chemical shift δ in ppm, coupling constant *J* in Hz: δ_H 1.42, 1.77 (2H, *m*, H-1), 1.10, 1.50 (2H, *m*, H-2), 1.78-1.87 (1H, *m*, H-3), 1.69-1.77 (1H, *m*, H-4), 0.12 (1H, *t*, *J* = 9.6 Hz, H-5), 0.62 (H, *ddd*, *J* = 6.4, 7.4 and 11.0 Hz, H-7), 1.24, 1.42 (2H, *m*, H-8), 1.38, 1.61 (2H, *m*, H-9), 1.61-1.69 (1H, *m*, H-11), 0.79, 1.42 (2H, *m*, H-12), 1.96-2.01 (2H, *m*, H-13), 5.01(1H, *t*, J = 7.2 Hz, H-14), 1.56 (3H, *s*,H-16), 1.65 (3H, *s*,H-17), 0.858 (3H, *s*, H-18), 1.01(3H, *s*, H-19), 0.88 (3H, *d*, *J* = 6.8 Hz, H-20).

3α-acetyl-tirucallic acid: White powder (15 mg); soluble in $CHCl_{3;}$ *Rf* = 0.60 (ss_2, vanillin/H_2SO_4); IR υ_{cm-1}(KBr): 3376 (O-H), 2931 (C-H), 1730 (Ac), 1704 (COOH), 1657 (C=C), 1373 (C-O); ^{1}H NMR (400 MHz, CDCl3), chemical shift δ in ppm, coupling constant *J* in Hz: δ_H 1.25, 1.67 (2H, *m*, H-1), 1.60, 1.67 (2H, *m*, H-2), 4.67(1H, *t*, *J* = 4.8 Hz, H-3), 1.23 (1H, H-5), 1.42, 1.67 (2H, *m*, H-6), 1.95, 2.07(2H, *m*, H-7), 1.91, 2.01(2H, *m*, H-11), 1.37, 1.67 (2H, *m*, H-12), 1.32, 1.57(2H, *m*, H-15), 1.33, 0.95 (2H, *m*, H-16), 2.05 (1H, *m*, H-17), 1.27 (3H, *s*, H-18), 0.88 (3H, *s*, H-19), 2.37 (1H, *t*, *J* = 7.6 Hz, H-20), 1.95, 2.00 (2H, *m*, H-23), 5.11(1H, *t, J* = 6.8 Hz, H-24), 1.69 (3H, *s*, H-26), 1.60 (3H, *s*, H-27), 0.98 (3H, *s*, H-28), 0.92 (3H, *s*, H-29), 0.95 (3H, *s*, H-30), 2.08 (3H, *s*, H-32).

3-Oxo-tirucallic acid: White powder (20 mg); soluble in $CHCl_{3;}$ *Rf* = 0.35 (mobile phase: ss_2, vanillin/H_2SO_4); UV (λ_{max} nm, EtOH): 248; IR υ_{cm-1}(KBr): 3428 (O-H), 2931 (C-H), 1754 (Ac), 1705 (COOH), 1657 (C=C), 1373 (C-O); ^{1}H NMR (400 MHz, CDCl3), chemical shift δ in ppm, coupling constant *J* in Hz: δ_H 2.07 (2H, *m*, H-1), 2.48 (2H, *dd*, *J* = 4.0, 3.6 Hz, H-2), 1.29 (1H, *m*, H-5), δ 2.43 (1H, *m*, H-17), 0.83 (3H, *s*, H-18), 0.92 (3H, *s*, H-19), 2.5 (1H, *m*, H-20), 5.11(1H, *t*, *J* = 7.2 Hz, H-24), 1.69 (3H, *s*, H-26), 1.60 (3H, *s*, H-27), 1.06 (3H, *s*, H-28), 1.11(3H, *s*, H-29), 1.05 (3H, *s*, H-30), 1.270-1.720 (*m*, other CH_2 protons).

3α-acetyl-β-boswellic acid and 3-acetyl-α-boswellic acid: Mixture of white powder (10 mg); soluble in $CHCl_{3;}$ *Rf* = 0.25 (mobile phase: ss_2, vanillin/H_2SO_4); ratio (β:α):1.0:0.67; ^{1}H NMR (400 MHz, CDCl3), chemical shift δ in ppm, coupling constant *J* in Hz: δ_H 5.09 (1H, *t*, *J* = 6.8 Hz, H-11 of A-β-BA), 5.15 (1H, t, *J* = 3.2 Hz, H-11 of A-α-BA), 5.31(1H, *t*, *J* = 2.4 Hz, H-3 of A-β-BA), 5.21(1H, *t*, J = 2.8 Hz, H-3 of A-α-BA), 1.869-2.024 (m, H-1 and H-$2_{\alpha/\beta}$), 1.25(3H, s, H-23_β), 0.94 (3H, *s*, H-25_β), 1.05 (3H, s, H-26_β), 1.13 (3H, *s*, H-27_β), 0.83 (3H, *s*, H-28_β), δ 0.82 (3H, *d*, *J* = 5.2 Hz, H-29_β), 0.92 (3H, *d*, H-30_β), 2.11 (3H, *s*, H-32_β), 1.20 (3H, *s*, H-23_α), 0.97 (3H, *s*, H-25_α), 1.01(3H, *s*, H-26_α), 0.85 (3H, *s*, H-27_α), 0.86 (3H, *s*, H-28_α), 0.90 (3H, *s*, H-29_α), 1.15 (3H, *s*, H-30_α), 2.10 (3H, *s*, H-32_α), 1.349-1.505 (*m*, other CH_2 protons).

3*α*-acetyl-11-keto-β-boswellic acid (AKBA): Fine crystal (15 mg); mp (^{o}C): 261-265; soluble in $CHCl_3$; *Rf* = 0.36 (mobile phase: ss_2, vanillin/H_2SO_4); UV (λ_{max} nm, EtOH): 250; IR υ_{cm-1}(KBr): 3434 (O-H), 2916 (C-H), 1733 (Ac), 1724 (C=O), 1705 (COOH), 1644 (C=C), 1392 (C-O); ^{1}H NMR (400 MHz, CDCl3), chemical shift δ in ppm, coupling constant *J* in Hz: δ_H δ 2.55 (2H, dt, *J* = 13.2, 3.3 Hz, H-1), 2.26 (2H, *td*, *J* = 3.2 and 14.8 Hz, H-2), 5.32 (1H, *bt*, H-3), 1.36 (1H, *m*, H-5), 1.85 (2H, *m*, H-6), 1.65 (2H, *m*, H-7), 2.43(1H, *s*, H-9), 5.58(1H, *s*, H-12), 1.89 (2H, *m*, H-15), 2.10 (2H, *dt*, J = 8.0, 4.0 Hz, H-16), 1.50 (1H, *d*, H-18), 1.41(1H, *m*, H-19), 0.91 (1H, *m*, H-20), 1.41 (2H, *m*, H-21), 1.44 (2H, *m*, H-22), 1.2 (3H, *s*, H-23), 1.16 (3H, *s*, H-25), 1.26 (3H, *s*, H-26), 1.36 (3H, *s*, H-27), 0.84 (3H, *s*, H-28), 0.80 (3H, *d*, J = 6.4 Hz, H-29), 0.96 (3H, *d*, J = 2.0 Hz, H-30), 2.1 (3H, *s*, H-32).

β-Boswellic acid and α-Boswellic acid: Mixture of white powder (10 mg); soluble in $CHCl_3$; *Rf* = 0.30 (mobile phase: ss_2, vanillin/H_2SO_4); UV (λ_{max} nm, EtOH): 250; ratio (β:α): 2:1; ^{1}H NMR (400 MHz, CDCl3), chemical shift δ in ppm, coupling constant *J* in Hz: δ_H 4.10 (1H, s, H-3_β), δ 4.14 (1H, s, H-3_α), 5.16 (1H, *t, J* = 3.6 Hz, H-12_β), 5.2 (1H, *t, J* = 3.6 Hz, H-12_α), 3.51 (2H, s, $OH_{\beta/\alpha}$), 1.37(3H, *s*, H-23_β), 1.02 (3H, *s*, H-25_β), 1.06 (3H, *s*, H-26_β), 1.11(3H, *s*, H-27_β), 0.89 (3H, *s*, H-28_β), 0.82(3H, *d*, J = 6.0 Hz, H-29_β), 0.92(3H, *bd*, H-30_β).

3-acetyl-11-hydroxy-β-boswellic acid: White powder (5 mg); Soluble in $CHCl_3$; *Rf* = 0.25 (mobile phase: ss_2, vanillin/H_2SO_4); UV (λ_{max} nm, EtOH): 248.5; IR υ_{cm-1}(KBr): 3442

(O-H), 2923 (C-H), 1708 (COOH), 1626 (C=C), 1240 (C-O); ^{1}H NMR (400 MHz, CDCl3), chemical shift δ in ppm, coupling constant *J* in Hz: δ_H 5.31(1H, *bt,* H-3), 2.15 (1H, *d*, *J* = 2.8, H-9), 4.28 (1H, *dd*, *J* = 9.0 and 2.8 Hz, H-11), 5.20 (1H, *d*, J = 2.8 Hz, H-12), 2.09 (1H, *m,* H-18), 2.01(1H, *dd, J* = 11.4 and 4.0 Hz, H-19), 1.85 (1H, *m*, H-20), 1.27 (3H, *s*, H-23), 1.08 (3H, *s*, H-25), 1.12 (3H, *s*, H-26), 1.23 (3H, *s*, H-27), 0.94 (3H, *s*, H-28), 0.82(3H, d, H-29), 0.87(3H, d, H-30), 2.12 (3H, *s*, H-32), 2.011-2.070 (2H, *m*, H-2), 1.330-1.890 (other CH_2 protons).

11-Keto-β-boswellic acid (KBA): White powder (5 mg); soluble in $CHCl_{3;}$ *Rf* = 0.25 (mobile phase: ss$_2$, vanillin/H_2SO_4); UV (λ_{max} nm, EtOH): 249.5; IR υ_{cm-1}(KBr): 3454 (O-H), 2921 (C-H), 1752 (C=O), 1708 (COOH), 1669 (C=C), 1235 (C-O); ^{1}H NMR (400 MHz, CDCl3), chemical shift δ in ppm, coupling constant *J* in Hz: δ_H 2.52 (2H, *dt*, *J* = 6.2 and 3.6 Hz, H-1), 2.28-2.37 (2H, *ddt, J* = 2.8, 1.2 and 8.8 Hz, H-2), 4.10 (*t*, *J* = 2.8 Hz, H-3), 1.42-1.49 (1H, *m*, H-5), 1.85-1.72 (2H, *m*, H-6), 1.65 (2H, *m*, H-7), 2.44 (1H, *s*, H-9), 5.56 (1H, *s*, H-12), 1.27-1.45 (2H, *m*, H-15), 1.00- 2.08 (2H, *m*, H-16), 1.51 (1H, *d*, *J* = 9.2 Hz, H-18), 1.39 (1H, *t*, *J* = 12.8 Hz, H-19), 0.96 (1H, *m*, H-20), 1.32 -1.44 (2H, *m*, H-21), 1.41(2H, *m*, H-22), 1.37 (3H, *s*, H-23), 1.15 (3H, *s*, H-25), 1.20 (3H, *s*, H-26), 1.33 (3H, *s*, H-27), 0.84 (3H, *s*, H-28), 0.82 (3H, *d*, *J* = 6.4 Hz, H-29), 0.96 (3H, *d*, *J* = 6.4 Hz, H-30).

4.4.2. Isolation of compounds from *B. papyrifera* resin (BpR)

A neutral fraction of *B. papyrifera* resin (2 g, yellow powder) (Section 4.2.3) was adsorbed in silica gel with methanol and subjected on normal phase column packed with silica gel (70 g, particle size 70-230 µm). Elusion has carried out with a gradient of solvents Hexane, $CHCl_3$ and EtOAc ratio with increasing polarity of the eluent to afford 14 fractions. Fraction 1 contains a mixture of monoterpenes, Fraction 3 was subjected to the small column and elusion was carried out with hexane to isolate verticilla-4(20), 7, 11-triene (**104,** BPK1**,** 150 mg). Fraction 8 was applied on the small column and elusion was carried out with a solvent gradient of Hexane: $CHCl_3$ to furnish incensole acetate (**111**, BPT2, 90 mg, clear oil). Fraction 11 was further subjected on the small column to isolate incensole (109, BPT3, 170 mg, clear oil) using Hexane: $CHCl_3$ as elusion solvent. Fraction 13 affords **166** and **169** after repeated column chromatography using hexane: CHCl$_3$: EtOAc as gradient elution solvents. The acid fraction of this resin (2 g, light yellow powder) was applied on a column. Elusion was carried out with same procedure to afford 15 fractions. Further purification of Fraction 1(200 mg) using small column furnished three compounds: fatty acid (**186**, BPK6, 5 mg), 3α-acetyl-tirucall-8, 24-din-21-oic acid **(**ATA, **169**, BPK7, 15 mg) and 3-oxo-tirucall-8, 24-dien-21-oic acid (3-OTA, **166**, BPK8, 20 mg). Fraction 2 (200 mg) was applied on a small column to isolate a mixture of 3α-acetyl-α-boswellic acid (AαBA, **147**, BPK9) and 3α-acetyl-β-boswellic acid (AβBA, **122**, BPK10). Fraction 3 (500 mg) was applied on a column followed purification using PTLC to obtain AKBA (**124**, BPK11, 15 mg), Fraction 4 (200 mg) was applied on a column to obtain AKBA (**124**, BPK11, 15 mg), a mixture of α-BA (**146**, BPK13) and β-BA (**121**, BPK12). Fraction 5 (150 mg) was fractionated in the same way to afford 11-OH-BA (**132**, BPK14, 5 mg) and KBA (**123**, BPK15, 5 mg).

Incensole acetate: Colorless viscous oil (100 mg); soluble in CHCl$_{3;}$ *Rf* = 0.65 (mobile phase: ss$_1$, vanillin/H$_2$SO$_4$); IR υ_{cm-1}(KBr): 2979 (C-H), 1743 (Ac), 1657 (C=C), 1383 (C-O); ^{1}H NMR (400 MHz, CDCl$_3$), chemical shift δ in ppm, coupling constant *J* in Hz: δ_H 1.75,1.62 (2H, *m*, H-2), 1.89,1.57 (2H, *m*, H-3), 4.88 (1H, *d, J* = 10 Hz, H-5), 1.85,1.51 (2H, *m*, H-6), 2.05, 1.64 (2H, *m*, H-7), 5.16(1H, bs, H-9), 2.19 (2H, *m*, H-10), 2.16 (2H, *m*, H-11), 5.16(1H, *bs,* H-13), 2.17(2H, *dd, J* = 5.6, 7.2 Hz, H-14), 1.10 (3H, *s*, H-15), 1.58 (3H, *s*, H-16), 1.54 (3H, *s*, H-17), 1.89 (1H, *m*, H-18), 0.89 (3H, *d*, *J* = 4 Hz, H-19), 0.91 (3H, *d*, *J* = 3.6 Hz, H-20), 2.06 (3H, *s*, H-22).

Incensole: Yellowish viscous oil (100 mg); soluble in CHCl$_{3;}$ *Rf* = 0.41(mobile phase: ss$_1$, vanillin/H$_2$SO$_4$); IR υ_{cm-1}(KBr): 3409 (O-H), 2951 (C-H), 1695 (C=C), 1373 (C-O); ^{1}H NMR (400 MHz, CDCl$_3$), chemical shift δ in ppm, coupling constant *J* in Hz: δ_H 1.59, 1.84 (2H, *m*, H-2), 1.74, 2.05 (2H, *m*, H-3), 3.27 (1H, *d*, *J* = 10 Hz, H-5), 1.32,1.88 (2H, *m*, H-6), 2.14, 1.99 (2H, *m*, H-7), 5.05 (2H, *m*, H-9, H-13), 2.10, 2.19 (2H, *m*, H-10), 2.10, 2.16 (2H, *m*, H-11), 2.03, 2.19 (2H, *m*, H-14), 1.06 (3H, *s*, H-15), 1.62 (3H, *s*, H-16), 1.49 (3H, *s*, H-17), 1.91 (1H, *m*, H-18), 0.89 (3H, *d*, *J* = 6.8 Hz, H-19), 0.90 (3H, *d*, *J* = 6.8 Hz, H-20).

4.4.3. Isolation of compounds from *B. sacra* (*Syn. B. carteri*) resin

A neutral fraction of *B. sacra* (*Syn. B. carteri*) resin (4 g, BsaRn) was applied on a column packed with silica-gel (70 g, mesh size 70-230 µm). Elusion was carried out with gradient of solvents Hexane: $CHCl_3$: EtOAc ratio with increasing polarity of the eluent to afford 15 fractions. Fraction 1 was contained a mixture of essential oils. 300 mg of Fraction 4 (that contain a major spot) was applied on small column to obtain serratol (**3**, Bsa3, 60 mg) which is marker spot for *B. sacra* and *B. serrata* in common. Fraction 5 (200 mg) was applied on column to obtain a mixture of ATA and 3-OTA. 4 g of acid fraction of this resin (BsaRa) was applied on a column to identified boswellic acids. Fraction 6 and Fraction 7 were containing boswellic acids (ABA, AKBA, BAs and KBA), their presence was confirmed by NMR figure print.

Serratol: Yellowish viscous oil (60 mg); soluble in $CHCl_{3;}$ *Rf* = 0.56 (mobile phase: ss_1, vanillin/H_2SO_4); IR υ_{cm-1}(KBr): 3473 (O-H), 2941 (C-H), 1657 (C=C), 1375 (C-O); ^1H NMR (400 MHz, CDCl3), chemical shift δ in ppm, coupling constant *J* in Hz: δ_H 2.14, 2.20 (2H, *m*, H-2), 5.25 (1H, *t*, J = 7.6 Hz, H-3), 2.21(2H, *m*, H-5), 2.30 (2H, *m*, H-6), 4.90 (1H, *t*, J = 6.4 Hz, H-7), 1.95, 2.11 (2H, *m,* H-9), 2.09 (2H, *m,* H-10), 5.00 5.00 (1H, *t*, J = 6.8 Hz, H-11), 1.75, 1.93 (2H, *m,* H-13), 1.65 (2H, *m,* H-14), 1.71 (1H, *m*, H-15), 1.55 (3H, *s*, H-16), 1.57 (3H, *s*, H-17), 1.59 (3H, *s*, H-18), 0.96 (3H, *d*, *J* = 6.4 Hz, H-19), 0.92 (3H, *d*, J = 7.2 Hz, H-20).

4.4.4. Isolation of compounds from *B. frereana* resin

A neutral fraction of *B. frereana* resin (4 g, BfRn) was applied on a column packed with silica-gel (70 g, mesh size 70-230 µm). Elusion was carried out with gradient of solvents Hexane: $CHCl_3$: EtOAc ratio with increasing polarity of the eluent to furnished 14 fractions. Fraction 1 and Fraction 2 contain one major spot each, Fraction 3 contain two major spots. Fraction 1 was purified using small column to isolate α-phellandrene dimer (**118**, 90 mg). Fraction 2 (200 mg) was applied on small column packed with silica-gel (12 g) to isolate a mixture of epilupeol acetate (**163**) and α-amyryl acetate (**191**, 5 mg). Fraction 3 (340 mg) was applied on a column to furnish 5 fractions of which Fraction 2 and Fraction 3 were identified as epilupeol (**158**, 200 mg) and lupeol (**159**, 50 mg, using PTLC).

α-Phellandrene dimer: Yellowish viscous oil (90 mg); soluble in $CHCl_3$; *Rf* = 0.71 (mobile phase: ss_1, vanillin/H_2SO_4); IR υ_{cm-1}(KBr): 2961 (C-H), 1665 (C=C), 1471; ^1H NMR (400 MHz, CDCl3), chemical shift δ in ppm, coupling constant *J* in Hz: δ_H 1.48-1.53, (4H, m, H-1/10), 1.98-2.02 (2H, *m*, H-2/9), 5.58 (2H, *dd*, *J* = 2.8, 7.6 Hz, H-3/8), 5.71 (1H, *d*, *J* = 10.4 Hz H-4/7), 1.96 (2H, *m*, H-11/12), 1.57-1.62 (2H, *m*, H-13/16), 0.92 (6H, *d, J* = 2.8 Hz, H-14/18), 0.90 (6H, *d, J* = 2.4 Hz, H-15/17), 0.94 (6H, *s*, H-19/20).

Epilupeol acetate and α-amyryl acetate: Mixture of white powder (5 mg); soluble in $CHCl_3$; *Rf* = 0.70 (mobile phase: ss_1, vanillin/H_2SO_4); UV (λ_{max} nm, EtOH): 250; ratio (1:0.3); IR υ_{cm-1}(KBr): 2951 (C-H), 1734 (Ac), 1638 (C=C), 1247 (C-O); ^1H NMR (400 MHz, CDCl3), chemical shift δ in ppm, coupling constant *J* in Hz: δ_H 4.62(1H, *bt*, H-3), 2.39 (1H, *td*, *J* = 5.6 and 4.8 Hz, H-19), 1.93 (1H, *m*, H-21), 2.06 (2H, *m*, H-18), 1.84-1.98 (1H, *m*, H-13), 1.05(*s*, H-23), 0.85(*s*, H-24), 0.80 (*s*, H-25), 1.01(*s*, H-26), 0.88(*s*, H-27), 0.84(*s*, H-28), 4.70(1H, *d*, J = 1.6 Hz, H-29β), 4.58(1H, *s*, H-29α), 1.69(*s*, H-30) and 2.09(*s*, H-32).

α-Amyrol acetate: ^1H NMR: 5.15 (1H, *t, J* = 3.6 Hz, H-12), 4.65 (1H, *bt*, H-3).

Epilupeol: White powder (200 mg); mp (^oC): 202-206; soluble in $CHCl_3$; *Rf* = 0.54 (mobile phase: ss_1, vanillin/H_2SO_4); UV (λ_{max} nm, EtOH): 250; IR υ_{cm-1}(KBr): 3496 (O-H), 2941 (C-H), 1636 (C=C), 1237 (C-O); ^1H NMR (400 MHz, CDCl3), chemical shift δ in ppm, coupling constant *J* in Hz: δ_H 3.40 (1H, t, H-3), 2.39 (1H, *td, J* = 6.0, 4.8 Hz, H-19), 1.94 (1H, *m*, H-21), 0.97 (3H, *s*, H-23), 0.80 (3H, *s*, H-24), 0.86 (3H, *s*, H-25), 1.05 (3H, *s*, H-26), 0.95 (3H, *s*, H-27), 0.84 (3H, *s*, H-28), 4.56 (1H, *d, J* = 2.0 Hz, H-29β) and 4.70 (1H, *d*, *J* = 2.0 Hz, H-29α), 1.70 (3H, s, H-23), 1.94-1.98 (2H, *m*, H-13, 20), 1.48, 1.38-1.43, 1.35, 1.18, 1.27 (*m*, CH2 protons).

Lupeol: White crystal (50 mg); MF: $C_{30}H_{50}O$; mp (^oC): 214-217; soluble in $CHCl_3$; *Rf* = 0.43 (mobile phase: ss_1, vanillin/H_2SO_4); UV (λ_{max} nm, EtOH): 272; IR υ_{cm-1}(KBr): 3321 (OH), 2941 (C-H), 1646 (C=C), 1373 (C-O); ^1H NMR (400 MHz, CDCl3), chemical shift δ in ppm, coupling constant *J* in Hz: δ_H 3.19 (1H, *dd*, *J* = 11.2, 4.8 Hz, H-3), 2.38 (1H, *td*, J = 5.2 and 5.6 Hz, H-19), 1.88-1.98(1H, m, H-21), 0.77 (3H, *s*, H-23), 0.80 (3H, *s*, H-24), 0.84 (3H, *s*, H-25), 0.96 (3H, *s*, H-26), 0.98 (3H, *s*, H-27), 1.04 (3H, *s*, H-28), 4.57 (1H, *d*, *J* = 2 Hz, H-29α), 4.68 (1H, *q*, H-29β), 1.69 (3H, *s*, H-30).

4.4.5. Isolation of compounds from *B. pirottae* resin

The EtOH crude extract of *B. pirottae* resin (2 g, yellowish jelly material) was adsorbed in silica gel with methanol and subjected on normal phase column packed with silica gel (70 g, particle size 70-230 µm). Elusion was carried out with gradient of solvents Pet-ether: CHCl$_3$ ratio with increasing polarity of the eluent to give 17 fractions. Fraction 4 (220 mg) was subjected to small column packed with silica gel (17 g) and eluted using pet-ether to collect 4 fractions of which Fraction 2 was applied on PTLC to isolate a mixture of eight fatty acid ester (Bpi2: **192-198**). Fraction 7 (300 mg) was applied on a small column to collect 7 fractions of which Fractions 2-4 were combined and applied on PTLC to isolate a mixture of four compounds (Bpi3): α-amyrone-**148**, β-amyrone-**149**, lupenone-**157** and tirucall-8,24-dienone-**200**, the same way Fractions 5-7 were recombined and applied on PTLC to isolate a mixture of three compounds (Bpi4): *epi-α*-amyrin-**130**, *epi-β*-amyrin-**149** and *3α*-OH-tirucall-8, 24-diene-**201**. Fraction 8 was applied on column and elusion was carried out with gradient of solvents Pet-ether: CHCl$_3$ with increasing polarity to collect series of fractions with same chemical profile. Fractions were recombined and applied on PTLC to isolate a clear spot identified as a mixture of four compounds (Bpi5): *α*-amyrin-**131**, β-amyrin-**150**, 3β-OH-tirucall-8, 24-diene-**159** and lupeol-**170**.

α-Amyrone, β-amyrone, lupenone and tirucall-8, 24-dienone: Yellow oil (60 mg); soluble in CHCl$_3$; *Rf* = 0.62 (mobile phase: ss$_1$, vanillin/H_2SO_4); UV (λ_{max} nm, EtOH): 272; ratio (α-Amyrone:β-Amyrone: Lupenone: Tirucallone): 1.0: 0.7: 0.4: 0.2; IR υ_{cm-1} (KBr): 2939 (C=C), 1713 (C=O), 1638 (C=C), 1475 (C-H, bending); ^{1}H NMR (400 MHz, CDCl3), chemical shift δ in ppm, coupling constant *J* in Hz: δ_H 5.19 (1H, *t, J* = 3.6 Hz, H-$12_{\alpha Am}$), 5.14 (1H, *t, J* = 3.2 Hz, H-$12_{\beta Am}$), 5.08 (1H, *t, J* = 6.8 Hz, H-24_T), 4.67 (1H, *d*, J = 2.0 Hz-H$29_{\beta L}$), 4.55 (1H, b*s,* H$29_{\alpha L}$), 2.40-2.42 (8H, *m*, H-2), 1.86-2.00 (8H, m, H-1), 2.34 (1H, m, H-19_L), 1.17-1.62 (*m*, CH_2 protons), 1.67 (6H, s, H-30_L, H-26_T), 0.76-1.13 (CH_3 protons).

Epi-α-amyrin, _epi-β_-amyrin and _3α_-OH-tirucall-8, 24-diene: Yellow oil (40 mg); soluble in $CHCl_3$; *Rf* = 0.54 (mobile phase: ss_1, vanillin/H_2SO_4); ratio (*e*-αAm: *e*-βAm: αT): 1.0: 0.4: 0.2; IR υ_{cm-1} (KBr): 3428 (O-H), 2939 (C-H), 1638 (C=C), 1375 (C-O), 1450 (C-H, bending), 1074 (C-O, bending); ^{1}H NMR (400 MHz, CDCl3), chemical shift δ in ppm, coupling constant *J* in Hz: δ_H 5.19 (1H, *t, J* = 3.2 Hz, H-$12_{\alpha Am}$), 5.14 (1H, *t, J* = 3.6 Hz, H-$12_{\beta Am}$), 5.11 (1H, *t, J* = 7.2 Hz, H-24_T), 3.42 (3H, H-$3_{\alpha,\beta Am,\alpha T}$), 1.77-2.09 (6H, *m*, H-$1_{\alpha,\beta Am,\alpha T}$), 1.53-1.71 (6H, m, H-$2_{\alpha,\beta Am,\alpha T}$), 1.24-1.44 (m, CH_2 protons), 0.79-1.10 (CH_3 protons).

_α_-Amyrin, _β_-amyrin and _3β_-OH-tirucall-8, 24-diene: Yellow oil (30 mg); soluble in $CHCl_3$; *Rf* = 0.43 (mobile phase: ss_1, vanillin/H_2SO_4); ratio (αAm:βAm: αT): 1.0: 0.4: 0.2; IR υ_{cm-1} (KBr): 3440 (O-H), 2939 (C-H), 1638 (C=C), 1370 (C-O), 1438 (C-H, bending), 1074 (C-O, bending); ^{1}H NMR (400 MHz, CDCl3), chemical shift δ in ppm, coupling constant *J* in Hz: δ_H 5.20 (1H, *t, J* = 3.6, H-$12_{\alpha Am}$), 5.14 (1H, *t, J* = 3.6, H-$12_{\beta Am}$), 5.10 (1H, *t, J* = 7.2, H-24_T), 4.70 (1H, *d*, *J* = 1.6 Hz, H-29_α), 4.58 (1H, *s,* H-29_β), 3.18 (1H, *dd, J* = 4.6, 6.4 Hz, H-3_L), 3.23 (1H, *dd, J = 5.2, 4.8 Hz, H-3*$_{\alpha,\beta Am,T}$), 1.69 (3H, s, H-30_L), 1.62 (3H, s, H-26_T), 1.88-2.41(*m*, H-1/H-2), 1.15-1.59 (the rest CH_2 and CH), 0.77-1.08 (the rest CH_3 protons).

4.4.6. Isolation of compounds from *B. neglecta* resin

A neutral fraction of *B. neglecta* resin (4 g, yellow powder, BnRn) was applied on a column packed with silica-gel (70 g, mesh 70-230 µm). Elusion was carried out with gradient of Hexane, $CHCl_3$ and EtOAc with increasing polarity of eluent to furnish 8 fractions. Fraction 5 was further applied on small column with same way to collect four fractions of which Fraction 1 was identified as a mixture of three compounds (BN2: **129, 148** and **157**) and Fraction 4 was identified as a mixture three compounds (BN3:**130, 148** and **158**). Fraction 8 was applied on PTLC to furnish a mixture of four compounds (BN4: **131, 150, 159** and 3-β-OH-tirucall-7,24-diene-**202**).

3β-OH-tirucall-7, 24-diene: Yellow oil; soluble in $CHCl_3$; *Rf* = 0.43 (mobile phase: ss_1, vanillin/H_2SO_4); ^{1}H NMR (400 MHz, $CDCl_3$), chemical shift δ in ppm, coupling constant *J* in Hz: δ_H 5.11(1H, *t, J* = 7.2 Hz, H-24), 5.27(1H, *t, J* = 3.2 Hz, H-7), 3.21 (1H, *dd,* J = 5.2, 6.0 Hz, H-3), 2.35-2.43 (2H, *m*, H-2), 1.81-2.06 (2H, *m*, H-1), 1.62 (3H, s, H-26), 1.27-1.58 (*m*, CH/CH_2 protons), 0.77-1.08 (CH_3 protons).

4.4.7. Isolation of compounds from *B. rivae* resin

The powdered resin *B. rivae* was extracted with EtOH over magnetic stirrer for 4 hours. The crude extract of this resin (4 g, yellowish jelly material) was adsorbed in the silica gel with methanol and subjected on a normal phase silica gel column (70 g, mesh 70-230). Elusion was carried out with a gradient of Pet-ether and $CHCl_3$ with increasing polarity of eluent to collect 31 fractions. Fraction 8 (300 mg) was applied on a column packed with silica gel (70 g), elusion was carried out the same way and 4 fractions were collected of which Fraction 1 and 2 were identified as (20S)-20-hydroxydammar-24-en-diol acetate (BR2, **203**) and 20S, 24S-epoxydammarane-25-ol-yl acetate (BR3, **204**), respectively. Fraction 12 (500) was applied on column packed with silica gel (70 g) elusion with same solvent gradient to collect 5 fractions of which Fraction 2 was identified as (20S)-20-hydroxydammar-24-en-3-one (BR4,**205**) and Fraction 4 was identified as (20S, 24S)-epoxydammaran-25-ol-3-one (BR5, **206**). Fraction 13 (320 mg) was applied on small column (17 g) and elusion was carried out with a gradient of Pet-ether: EtOAc with increasing polarity to collect 6 fractions of which Fraction 5 was identified as a mixture of two compounds: dammaranelactone-3α-acetate (BR6, **207**) and (3β, 20S)-dammar-24-endiol (BR7, **117**) with a ratio of 1:0.3, respectively.

(20S)-20-hydroxydammar-24-en-diol acetate: Colorless oil (25 mg); soluble in $CHCl_3$; *Rf* = 0.67 (mobile phase: ss_1, vanillin/H_2SO_4); UV (λ_{max} nm, EtOH): 238.5; IR υ_{cm-1}(KBr): 3465 (O-H), 2939 (C-H), 1713 (Ac), 1638 (C=C), 1362, 1237 (C-O), 1463 (C-H, bending), 1072b(C-O, bending); ^{1}H NMR (400 MHz, CDCl3), chemical shift δ in ppm, coupling constant *J* in Hz: δ_H 0.94 (3H, *s*, H-18), 1.18 (3H, *s*, H-19), 1.23 (3H, *s*, H-21), 1.70 (3H, *s*, H-26), 1.64 (3H, *s*, H-27), 1.16 (3H, *s*, H-28), 0.98 (3H, *s*, H-29), δ 0.88 (3H, *s*, H-30), 2.10 (3H, *s*, H-32), 4.64 (1H, *bs*, H-3), 5.14 (*t, J* = 6 Hz, H-24), 1.44-1.51 (2H, *m*, H-22), 2.05 (2H, *m*, H-23).

20S, 24S-epoxydammarane-25-ol-yl acetate: Colorless oil (40 mg); soluble in $CHCl_{3;}$ *Rf* = 0.61 (mobile phase: ss$_1$, vanillin/H_2SO_4); UV (λ_{max} nm, EtOH): 241; IR ʋ$_{cm-1}$(KBr): 3465 (O-H), 2964 (C-H), 1375, 1237 (C-O), 1450 (C-H, bending), 1174, 1026 (C-O, bending); ^{1}H NMR (400 MHz, CDCl3), chemical shift δ in ppm, coupling constant *J* in Hz: δ_H 1.44, 1.16 (2H, *m*, H-1), 1.87, 1.57 (2H, *m*, H-2), 4.62 (1H, t, J=2.8 Hz, H-3), 1.23 (1H, *m*, H-5), 1.45 (2H, *m*, H-6), 1.57, 1.26 (2H, *m*, H-7), 1.46 (1H, *m*, H-9), 1.52, 1.18 (2H, *m*, H-11), 1.80, 1.26 (2H, *m*, H-12), 1.64 (1H, *m*, H-13), 1.47, 1.08 (2H, *m*, H-15), 1.76, 1.30 (2H, *m*, H-16), 1.87 (1H, *m*, H-17), 0.98 (3H, *s*, H-18) 0.87 (3H, *s*, H-19), 1.15 (3H, *s*, H-21), 1.88, 1.68 (2H, *m*, H-22), 1.89, 1.77 (2H, *m*, H-33), 3.63 (1H, dd, J = 5.6, 4.4 Hz, H-24), 1.19 (3H, *s*, H-26), 1.12 (3H, *s*, H-27), 0.83 (3H, *s*, H-28), 0.88 (3H, *s*, H-29), 0.92 (3H, *s*, H-30), 2.08 (3H, *s*, H-31).

(20S)-20-hydroxydammar-24-en-3-one: Colorless oil (50 mg); soluble in $CHCl_{3;}$ *Rf* = 0.58 (mobile phase: ss$_1$, vanillin/H_2SO_4); UV (λ_{max} nm, EtOH): 239; IR ʋ$_{cm-1}$(KBr): 3465 (O-H), 2952 (C-H), 1700 (C=O), 1650 (C=C), 1375 (C-O); ^{1}H NMR (400 MHz, CDCl3), chemical shift δ in ppm, coupling constant *J* in Hz: δ_H 1.50, 1.97 (2H, *m*, H-1), 2.33-2.56 (2H, *m*, H-2), 1.42 (1H, dd, J = 8.2, 7.6 HZ, H-5), 1.54, 1.58 (2H, *m*, H-6), 1.37, 1.65 (2H, *m*, H-7), 1.48 (1H, *m*, H-9), 1.33, 1.56 (2H, *m*, H-11), 1.54, 1.83 (2H, *m*, H-12), 1.74 (1H, *m*, H-13), 1.14, 1.51 (2H, *m*, H-15), 1.33, 1.91 (2H, *m*, H-15), 1.83 (1H, *m*, H-17), 0.95 (3H, *m*, H-18), 1.01 (3H, *m*, H-19), 1.16 (3H, *m*, H-21), 1.54 (2H, *m*, H-22), 2.09 (2H, *m*, H-23), 5.13 (1H, *t,* J = 7.2 Hz, H-24), 1.70 (3H, *m*, H-26), 1.63 (3H, *m*, H-27), 1.09 (3H, *m*, H-28), 1.04 (3H, *m*, H-29), 0.89 (3H, *m*, H-30).

20S, 24S-epoxydammaran-25-ol-3-one: Colorless oil (70 mg); soluble in $CHCl_{3;}$ *Rf* = 0.50 (mobile phase: ss$_1$, vanillin/H_2SO_4); UV (λ_{max} nm, EtOH): 238; IR ʋ$_{cm-1}$(KBr): 3528 (O-H), 2964 (C-H), 1688 (C=O), 1375, 1325 (C-O), 1475 (C-H, bending), 1049 (C-O, bending); ^{1}H NMR (400 MHz, CDCl3), chemical shift δ in ppm, coupling constant *J* in Hz: δ_H 1.46, 1.94 (2H, *m*, H-1), 2.43-2.50 (2H, *m*, H-2), 1.36 (1H, *m*, H-5), 1.50, 1.56 (2H, *m*, H-6), 1.30, 1.58 (2H, *m*, H-7), 1.44 (1H, *m*, H-9), 1.26, 1.53 (2H, *m*, H-11), 1.32, 1.81 (2H, *m*, H-12), 1.68 (1H, *m*, H-13), 1.13, 1.50 (2H, *m*, H-15), 1.26, 1.85 (2H, *m*, H-16), 1.91 (1H, *m*, H-19), 0.94 (3H, *s*, H-18), 1.04 (3H, *s*, H-19), 1.15 (3H, *s*, H-21), 1.69, 1.91(2H, *m*, H-22), 1.87 (2H, *m*, H-23), 3.64 (1H, dd, J = 5.6, 4.4 Hz, H-24), 1.19 (3H, *s*, H-26), 1.11(3H, *s*, H-27), 1.08 (3H, *s*, H-28), 1.01(3H, *s*, H-29), 0.88 (3H, *s*, H-30).

Dammaranelactone-3α-acetate: Colorless oil (40 mg); soluble in $CHCl_{3;}$ *Rf* = 0.45 (mobile phase: ss_1, vanillin/H_2SO_4); UV (λ_{max} nm, EtOH): 241; IR υ_{cm-1}(KBr): 2952 (C-H), 1763, 1726 (C=O), 1375, 1250 (C-O), 1438 (C-H, bending); ^{1}H NMR (400 MHz, CDCl3), chemical shift δ in ppm, coupling constant *J* in Hz: δ_H 1.01, 1.75 (2H, *m*, H-1), 1.60, 1.69 (2H, *m*, H-1), 4.63 (1H, *bt*, H-3), 1.40 (1H, *m*, H-5), 1.56 (2H, *m*, H-6), 1.29, 1.57 (2H, *m*, H-1), 1.31 (1H, *m*, H-9), 1.26, 1.54 (2H, *m*, H-11), 1.87 (2H, *m*, H-2), 1.60 (1H, *m*, H-13), 1.17, 1.53 (2H, *m*, H-15), 1.26, 1.76 (2H, *m*, H-16), 2.00 (1H, *m*, H-17), 0.87 (3H, *s*, H-18), 0.94 (3H, *s*, H-19), 1.37 (3H, *s*, H-21), 1.96, 2.12 (2H, m, H-22), 2.51-2.69 (2H, *m*, H-23), 0.97 (3H, *s*, H-28), 0.89 (3H, *s*, H-29), 0.84 (3H, *s*, H-30), 2.09 (3H, *s*, H-32).

(3β, 20S)-Dammar-24-endiol: ^{1}H NMR and ^{13}C NMR (400 MHz, CDCl3), δ_H 1.00,1.70 (2H, *m*, H-1), 1.60 (2H, *m*, H-2), 3.20 (dd,J= 4.8, 6.4 Hz, H-3), 0.75 (1H, d, 10.4 Hz, H-5), 1.46, 1.51 (2H, *m*, H-6), 1.30, 1.55 (2H, *m*, H-7), 1.31 (1H, *m*, H-9), 1.27, 1.51 (2H, *m*, H-11), 1.50, 1.75 (2H, *m*, H-12), 1.62 (1H, *m*, H-13), 1.47, 1.07 (2H, *m*, H-15), 1.27, 1.82 (2H, *m*, H-16), 1.75 (1H, *m*, H-17), 0.86 (3H, *s*, H-18), 0.97 (3H, *s*, H-19), 1.15 (3H, *s*, H-21), 1.48 (2H, *m*, H-22), 2.05 (2H, *m*, H-23), 5.13 (1H, *t, J* = 6.8 Hz, H-24), 1.70 (3H, *s*, H-26), 1.64 (3H, *s*, H-27), 0.99 (3H, *s*, H-28), 0.79 (3H, *s*, H-29), 0.89 (3H, *s*, H-30).

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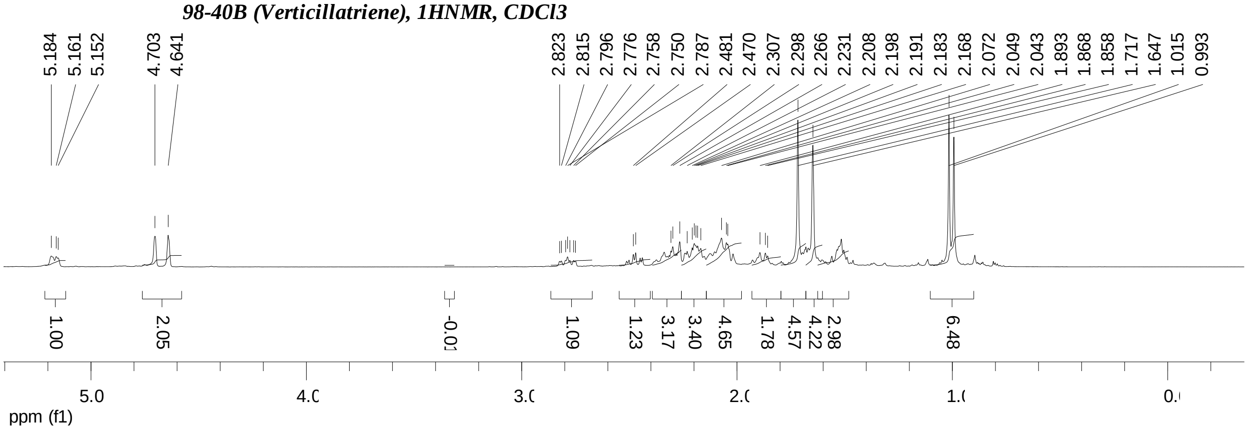
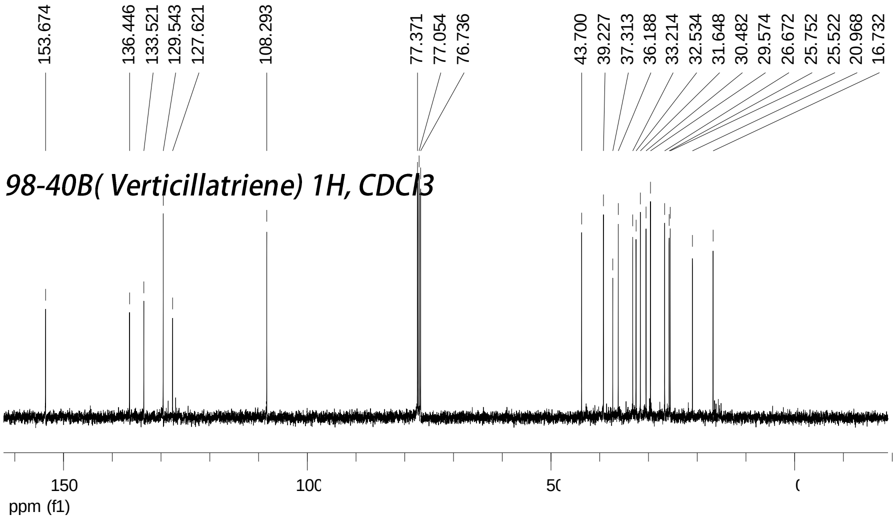
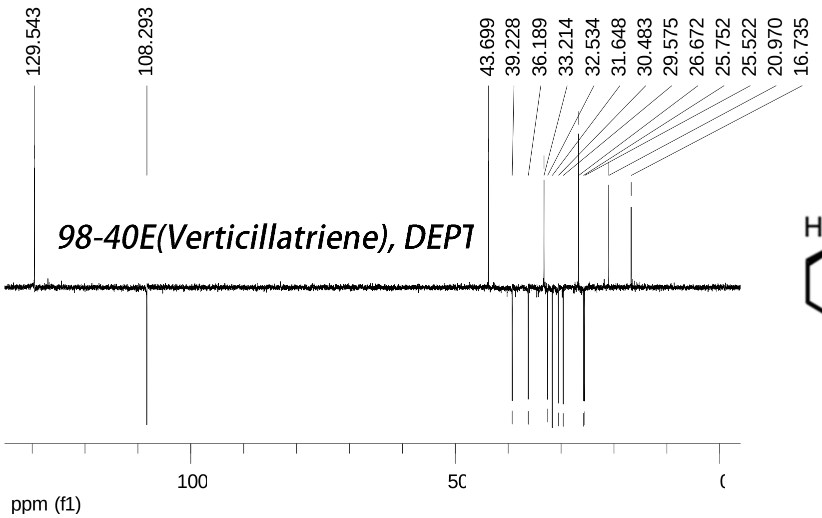
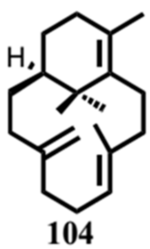
Appendixes

Appendix 1: NMR data of verticill-3,7(20),11-triene (**104**)



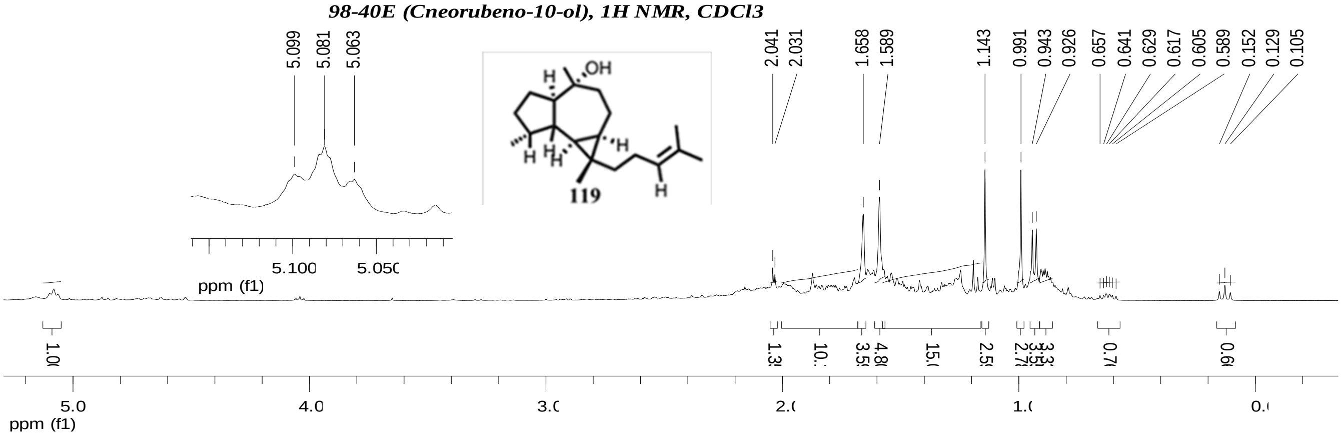




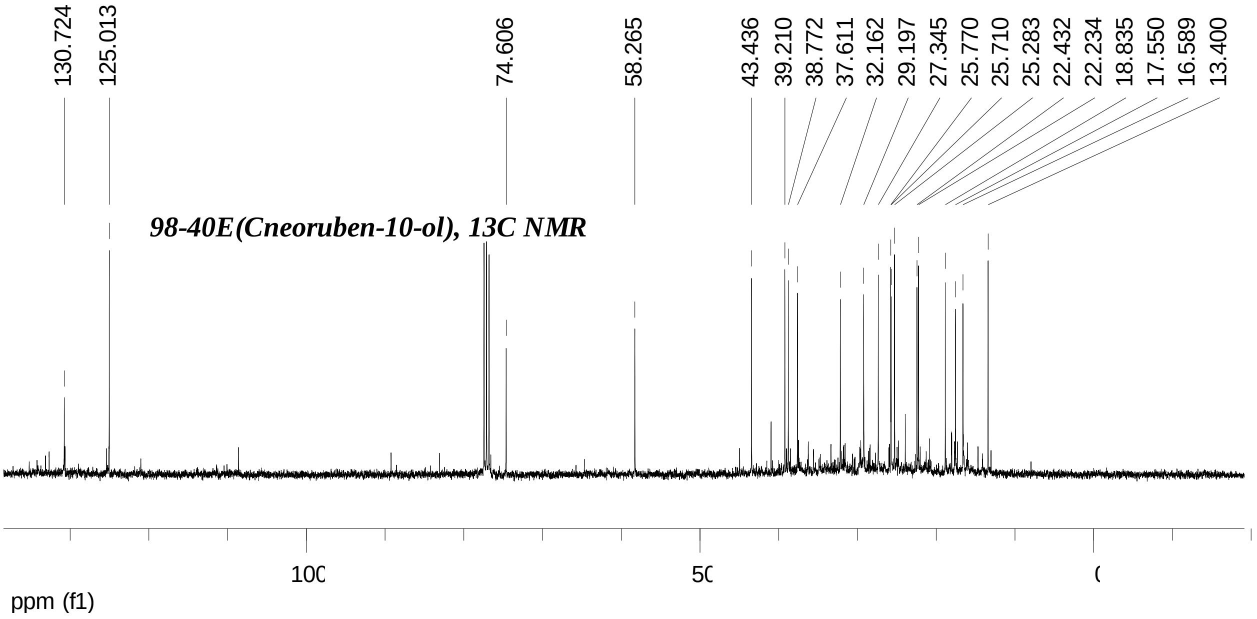


104

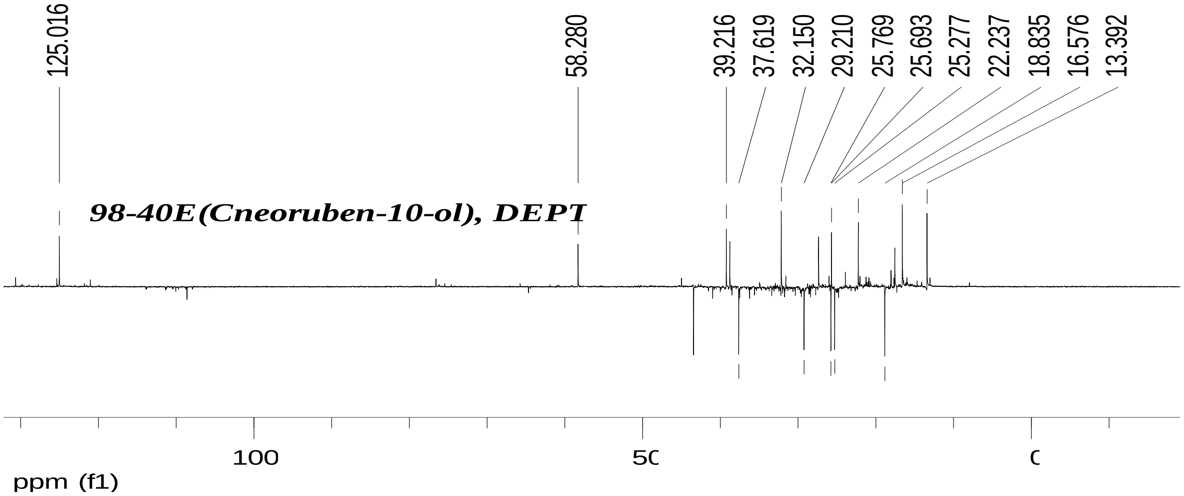
Appendix 2: NMR data of cneoruben-10-ol (**119**)



98-40E (Cneorubeno-10-ol), 1H NMR, CDCl3

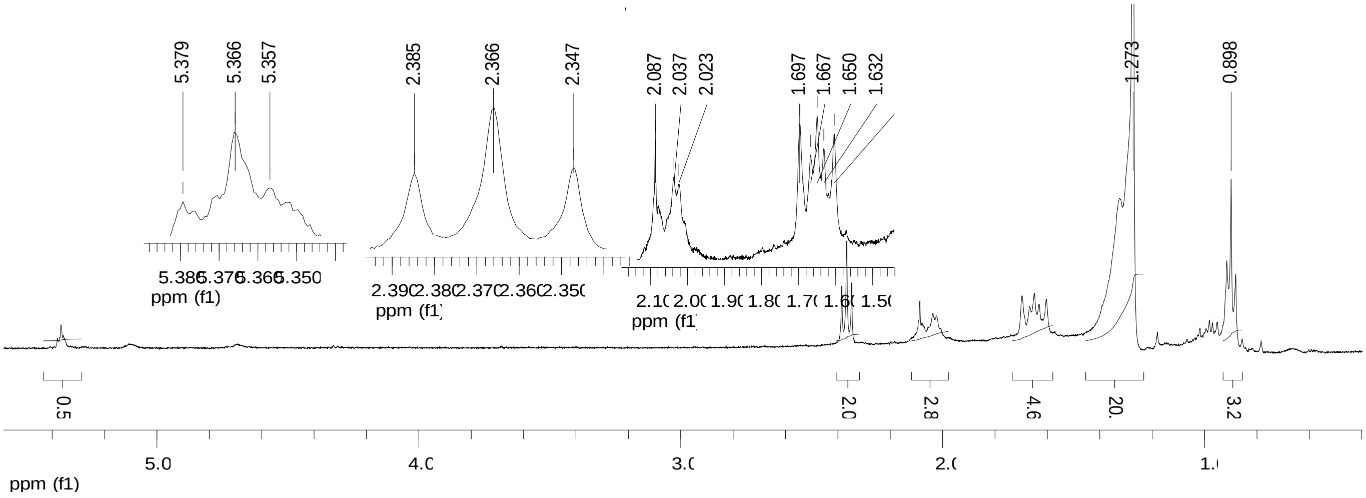


98-40E(Cneoruben-10-ol), 13C NMR

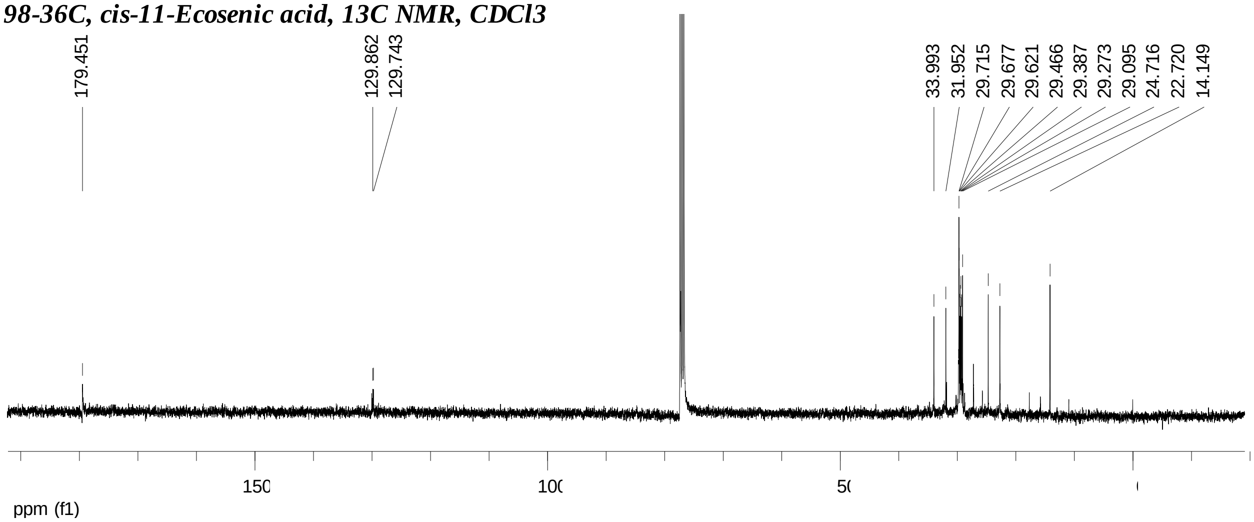


98-40E(Cneoruben-10-ol), DEPT

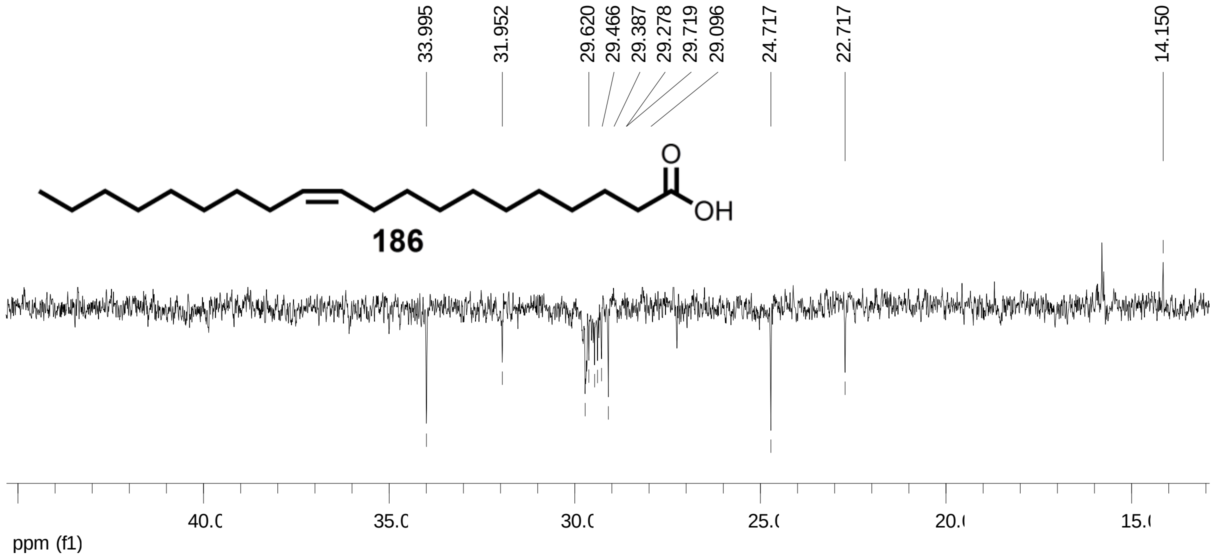
Appendix 3: NMR data of *cis*-11-eicosenoic acid (**186**)



98-36C, cis-11-Ecosenic acid, 13C NMR, CDCl3

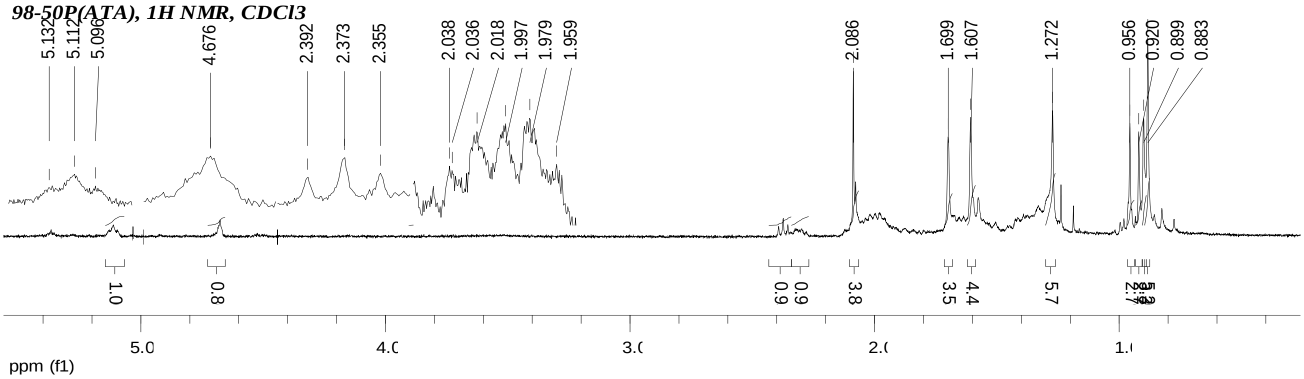


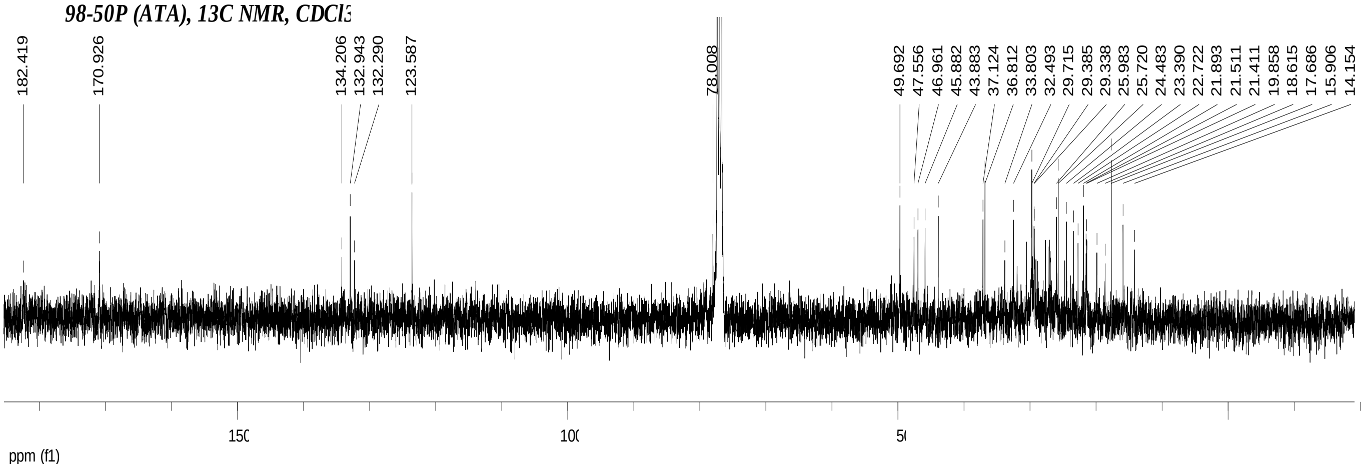
98-36C, cis-11-Ecosenic acid, DEPT, CDCl3

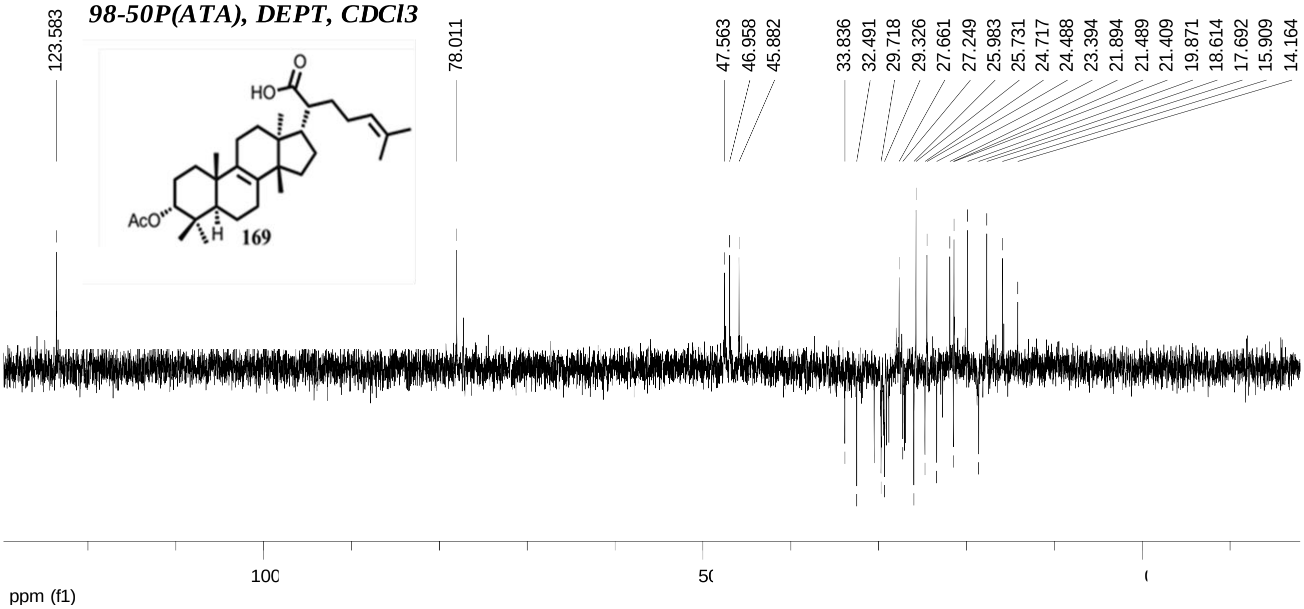


186

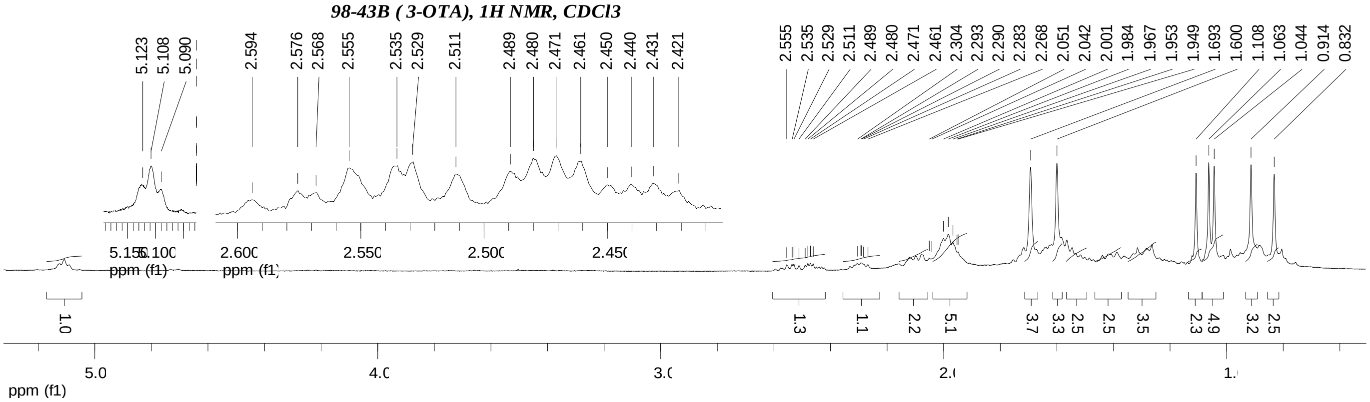
Appendix 4: NMR data of 3α-acetyl tirucall-8,24-dien-24-oic acid (**169**)

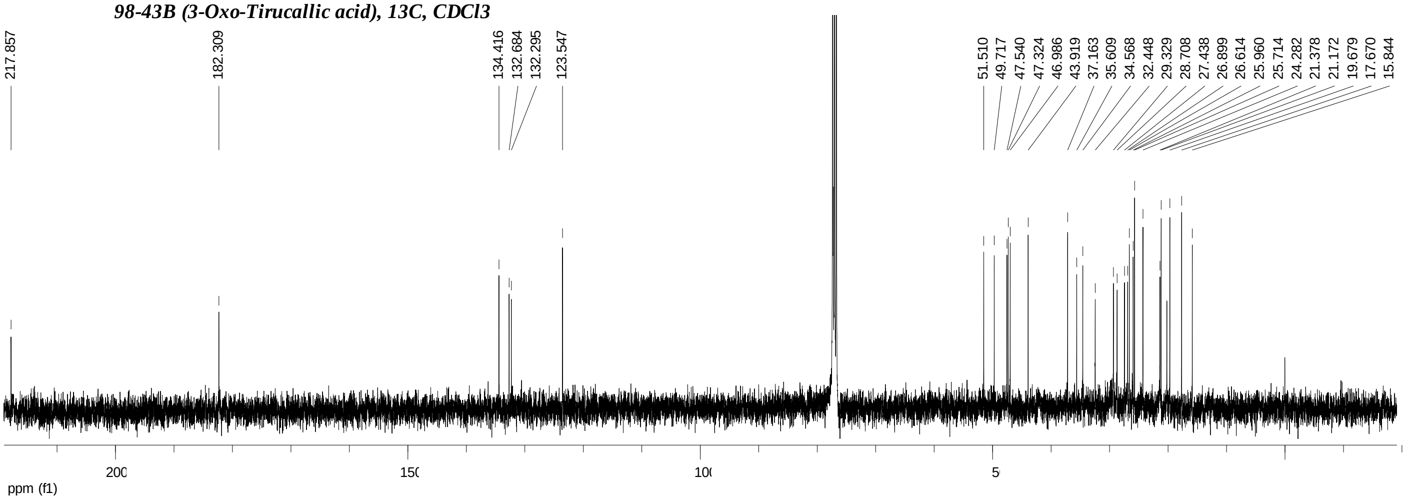

98-50P(ATA), 1H NMR, CDCl3

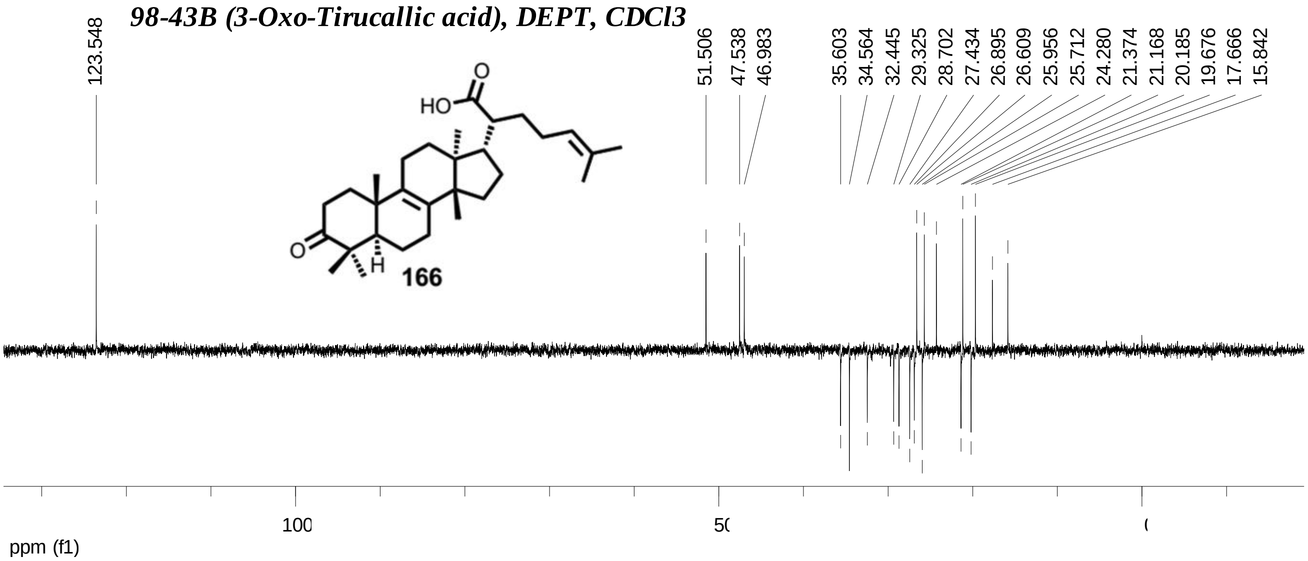

98-50P (ATA), 13C NMR, CDCl3


98-50P(ATA), DEPT, CDCl3

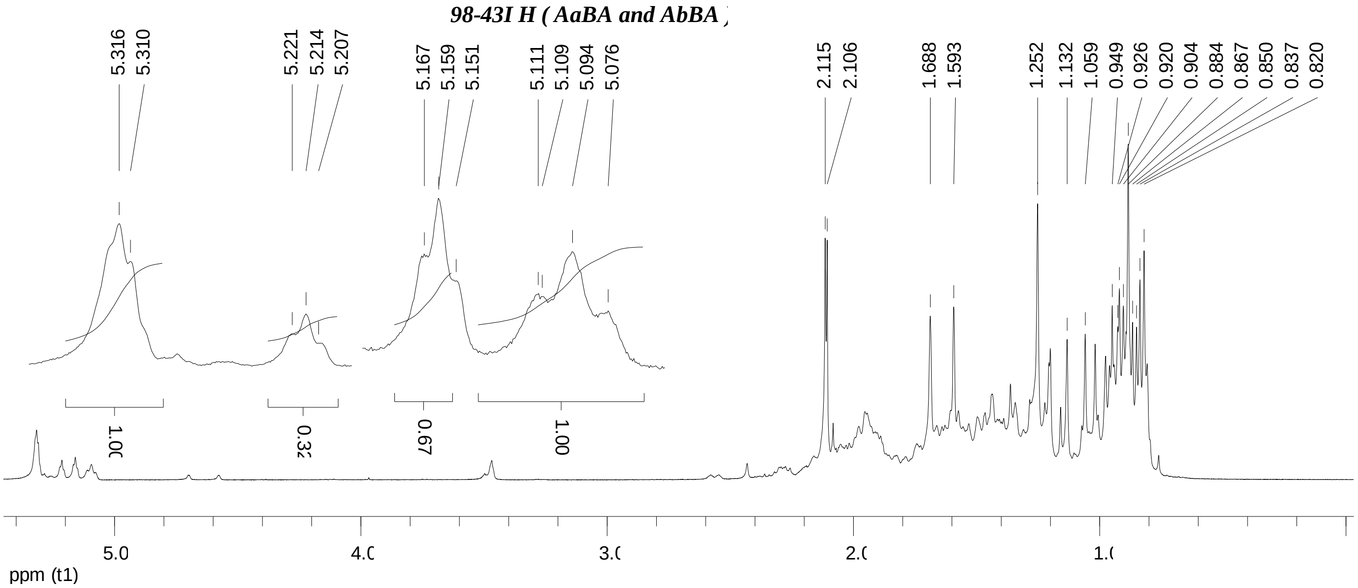
Appendix 5: NMR data of tirucall-8,24-dien-3-one (**166**)

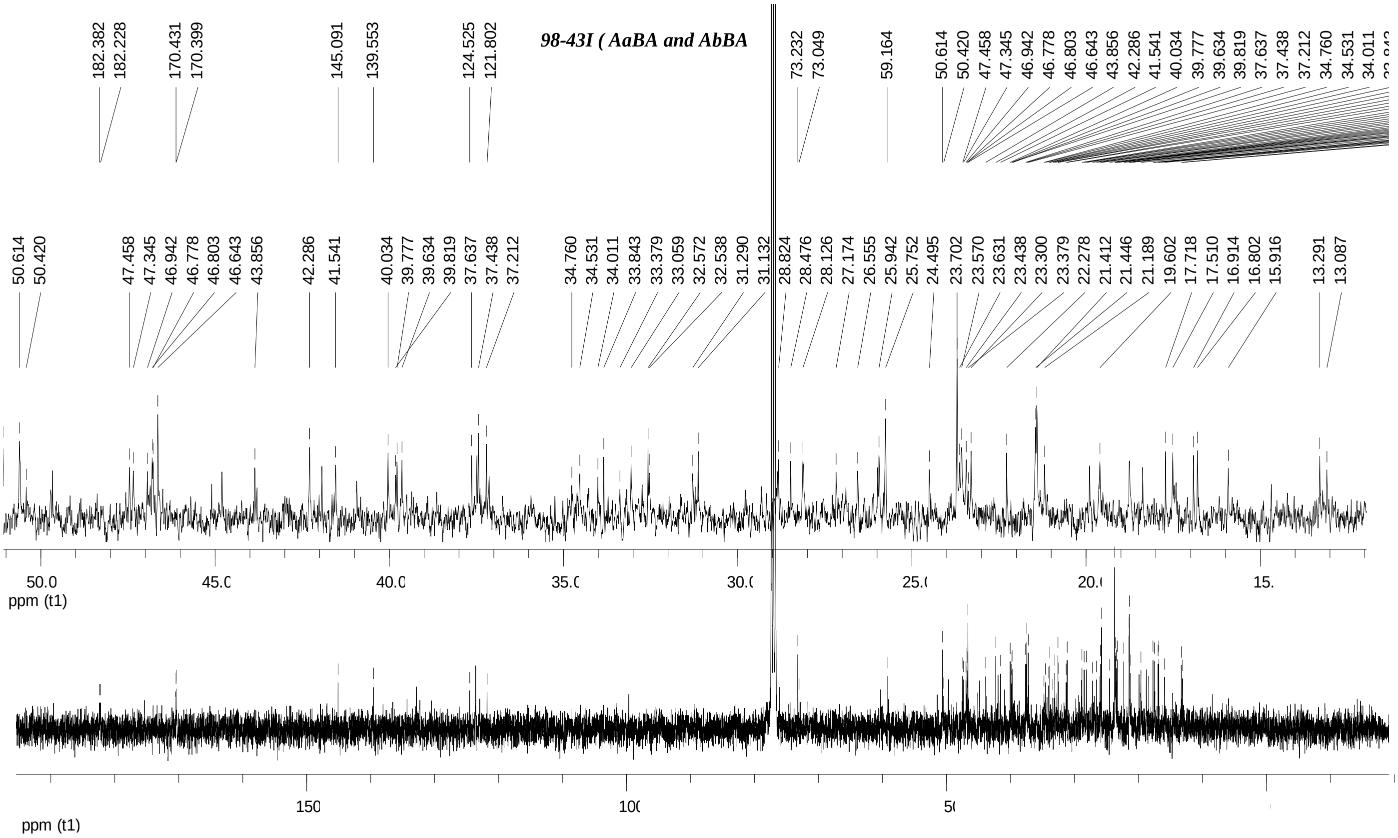


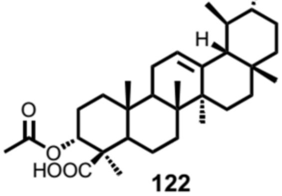




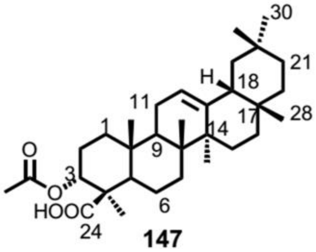
Appendix 6: NMR data of 3α-acetyl-α-boswellic acid (**122**) and 3α-acetyl-β-boswellic acid (**144**)





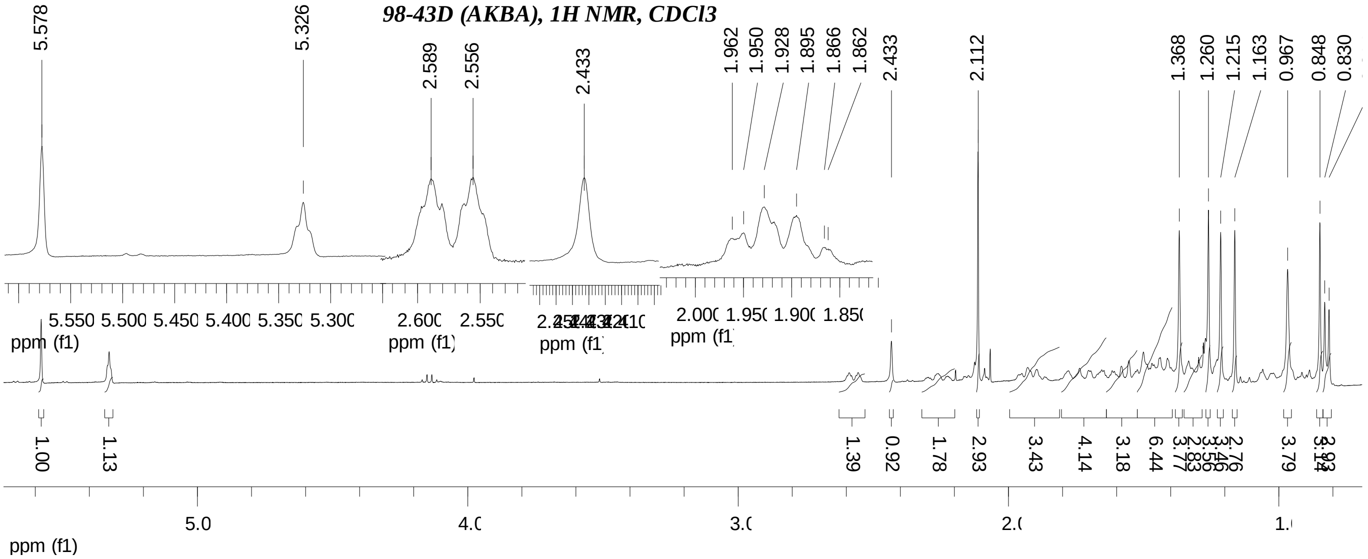


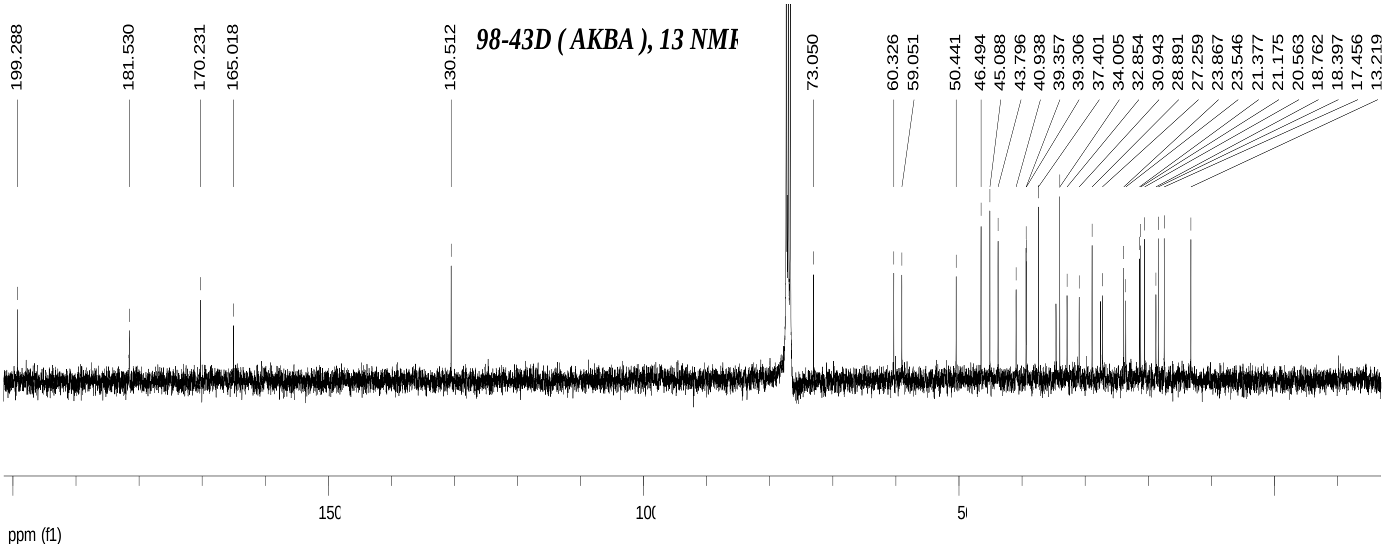
122

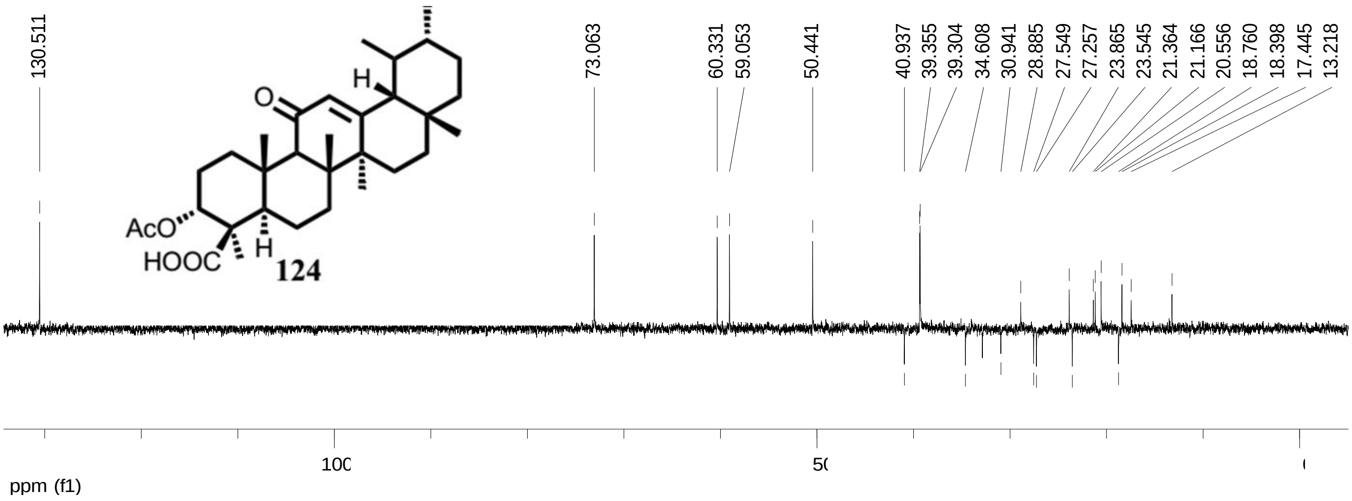


147

Appendix 7: NMR data of 3-acetyl-11-oxo-β-boswellic acid (**124**)

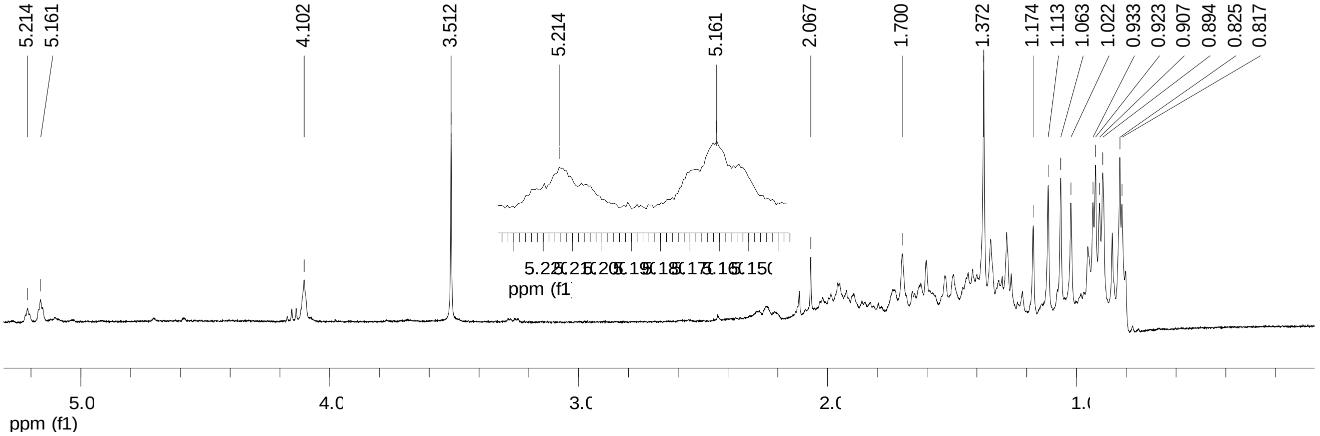




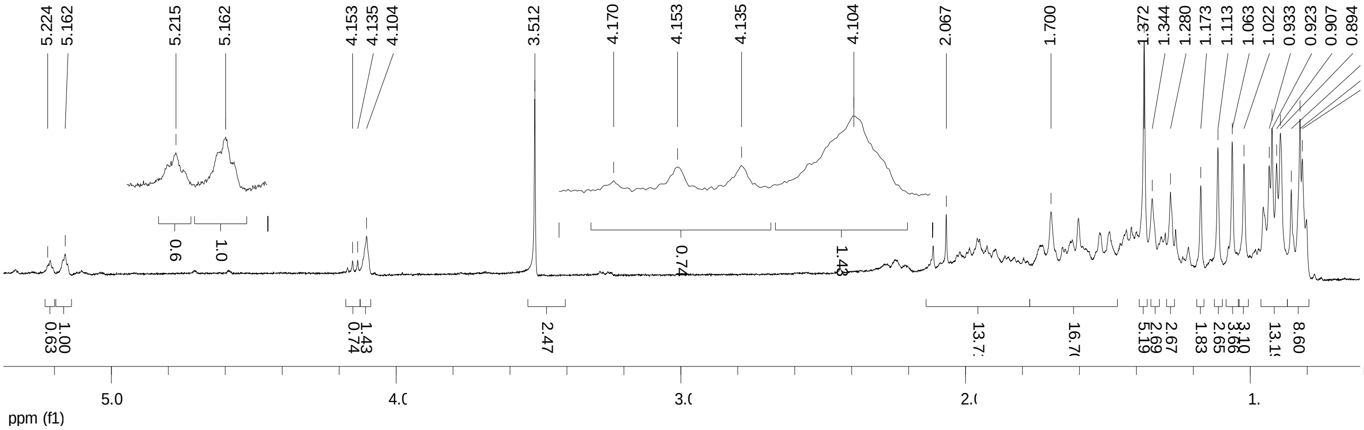


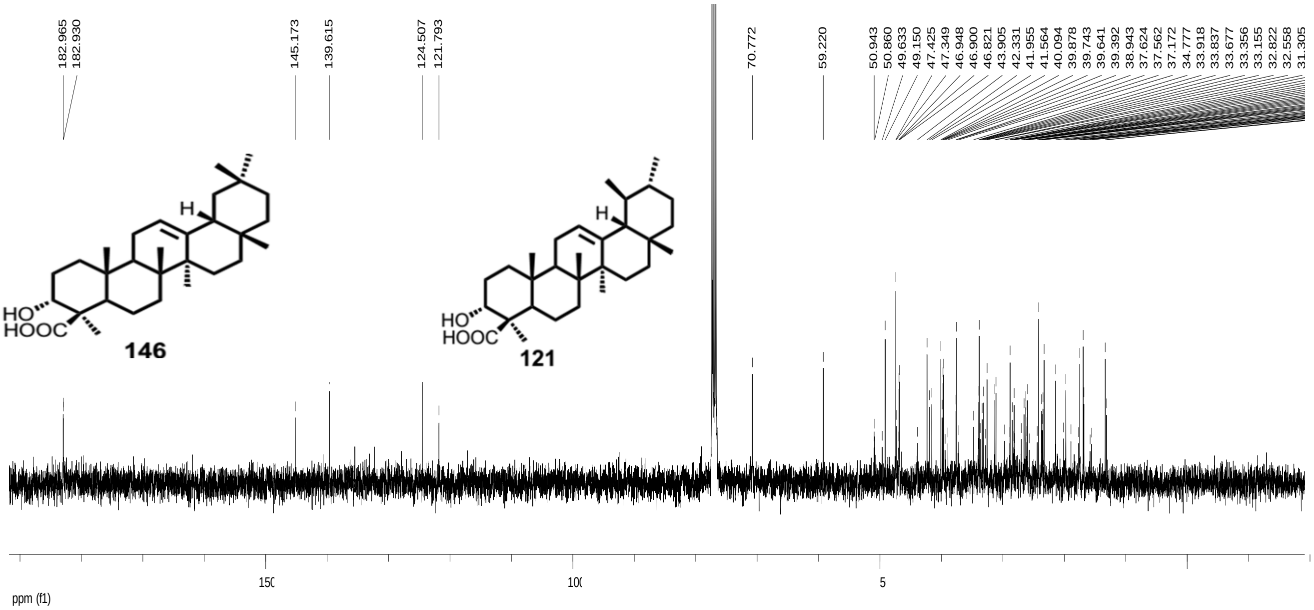
Appendix 8: NMR data of α-boswellic acid (**121**) and β-Boswellic acid (**146**)

98-43H (BAs), 1H NMR, CDCl3



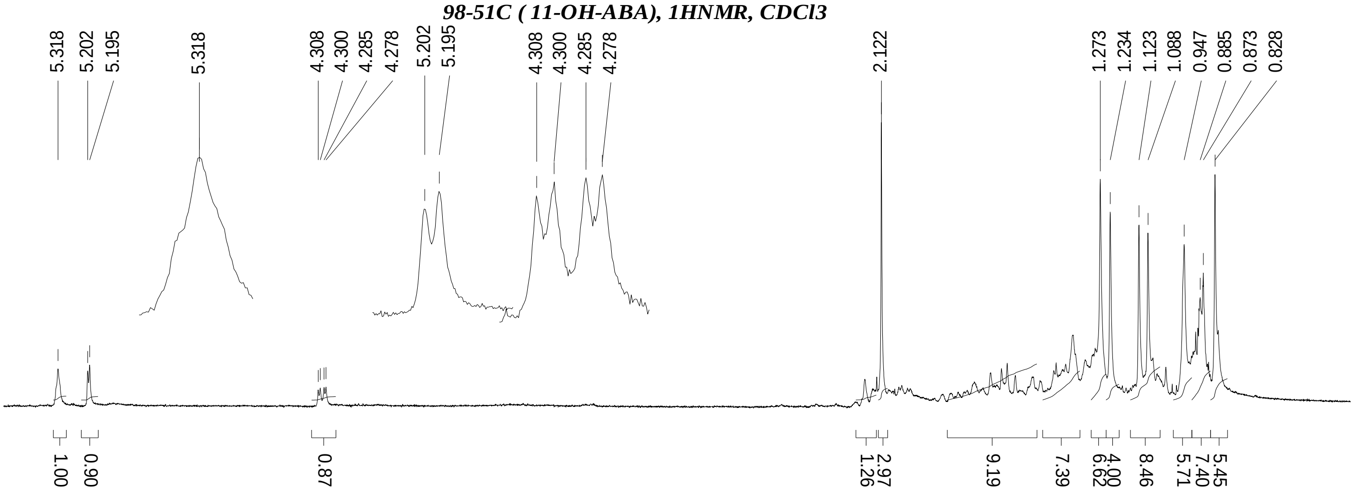
98-43F(BAs), 1H NMR, CDCL3,

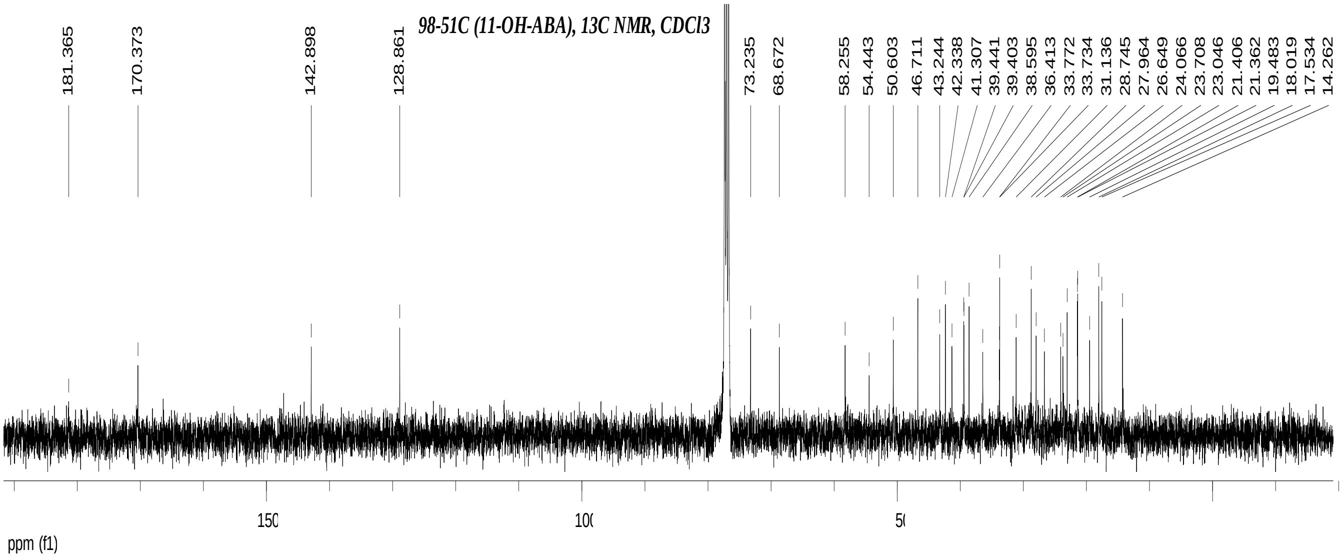


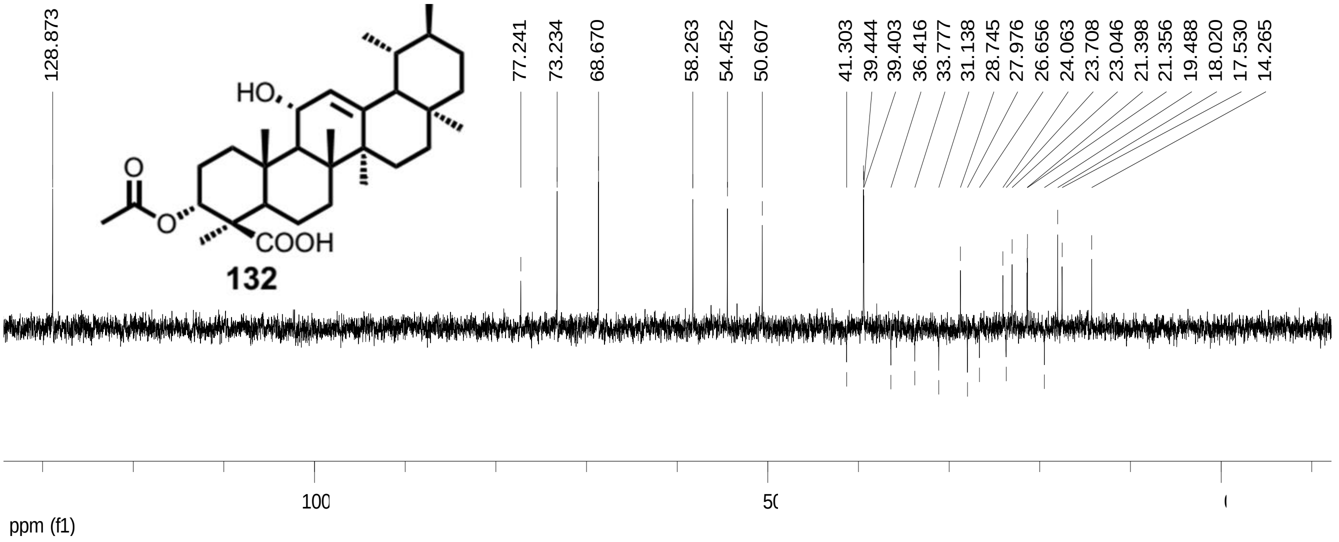


98-19 F

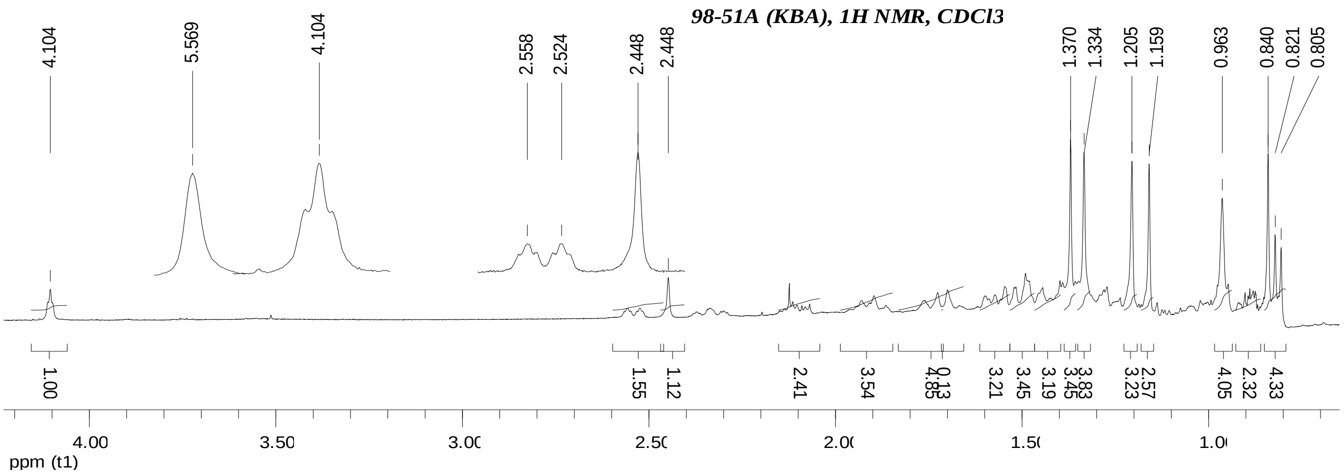
Appendix 9: NMR data of 11-Hydroxy-β-boswellic acid (**132**)

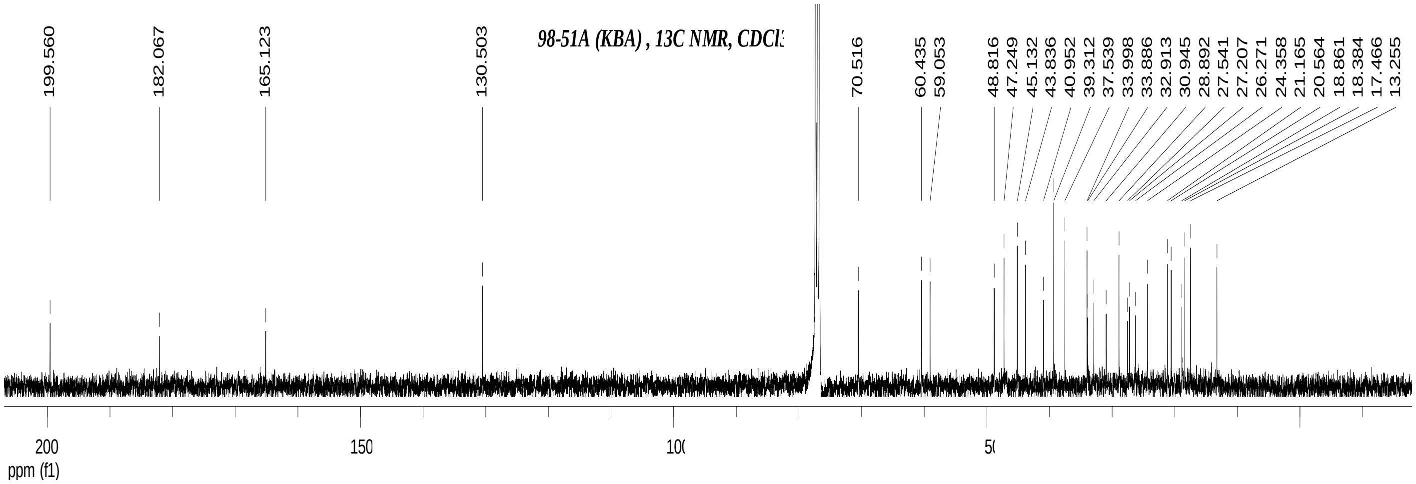


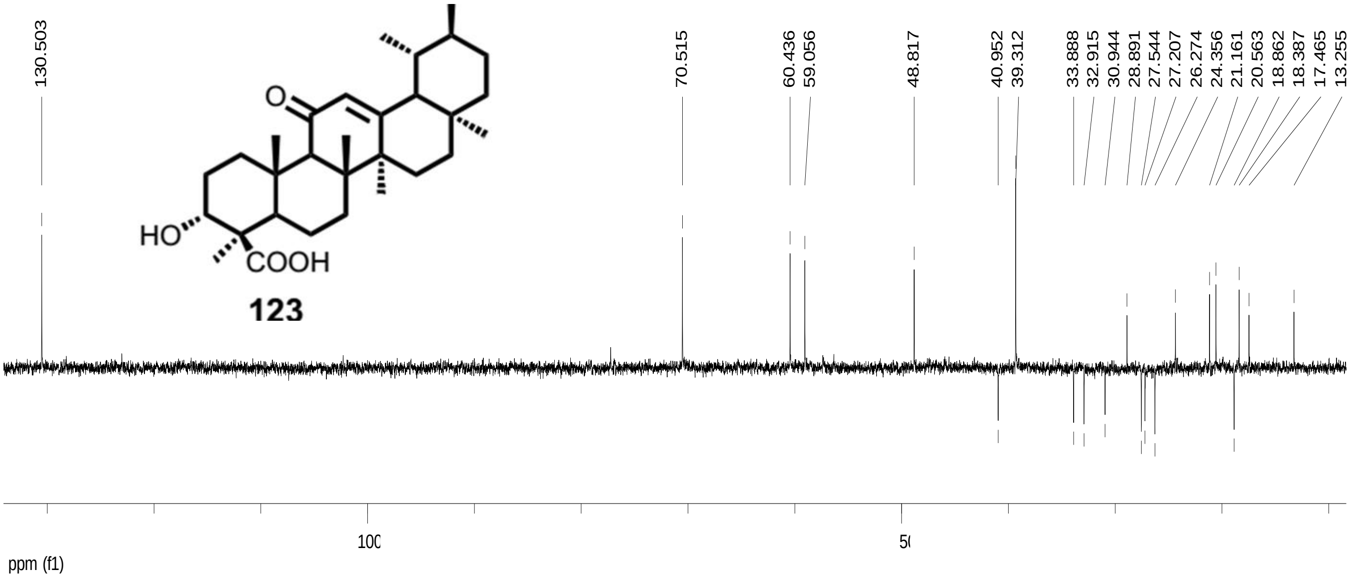




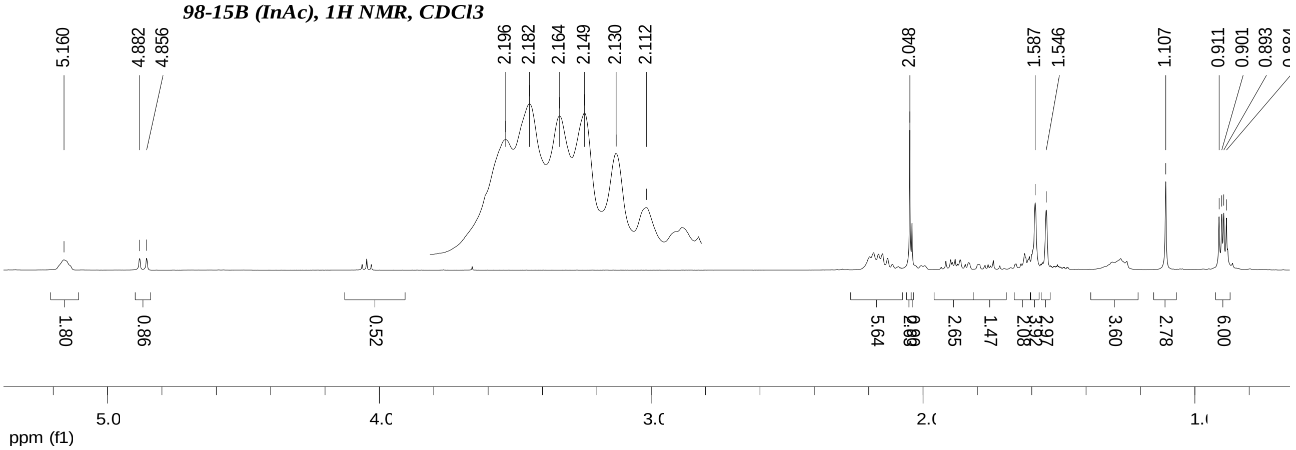
Appendix 10: NMR data of 11-keto-β-boswellic acid (**123**)

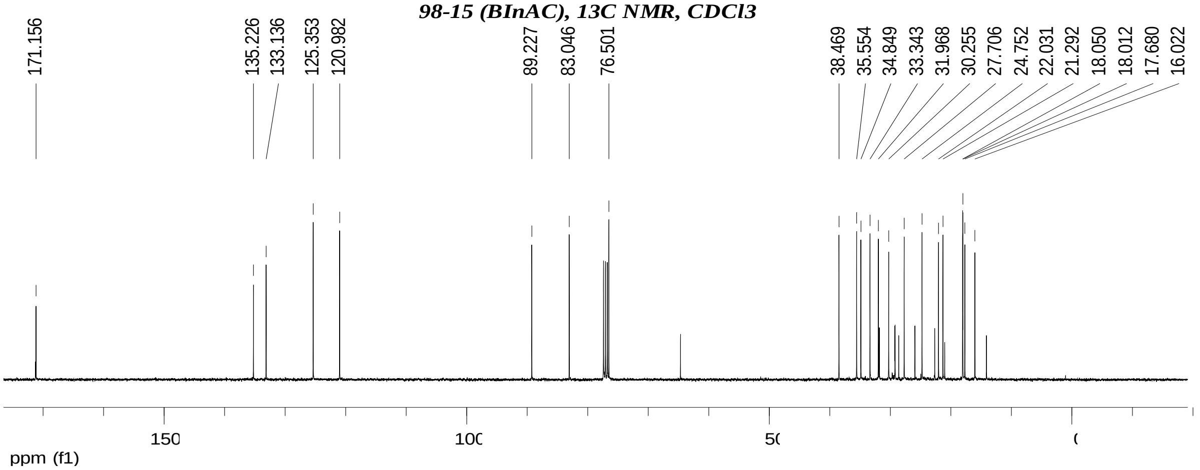


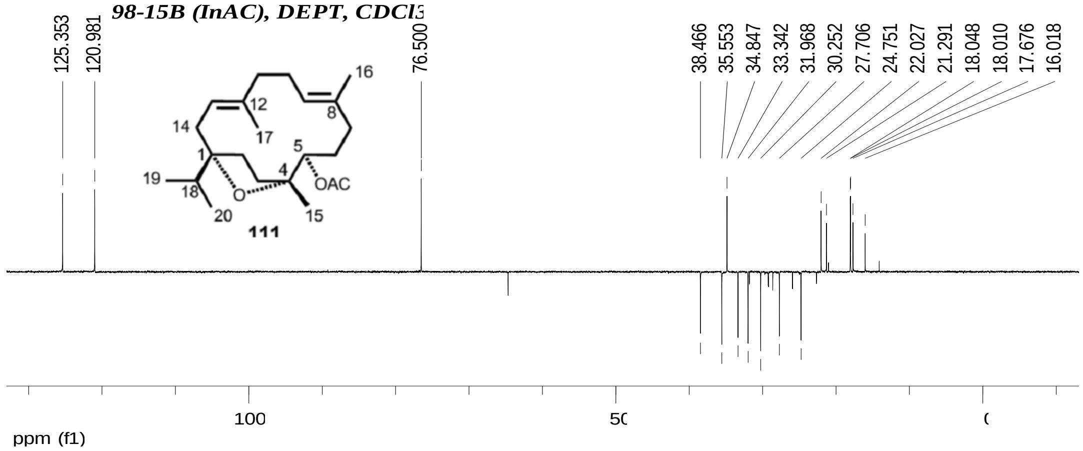




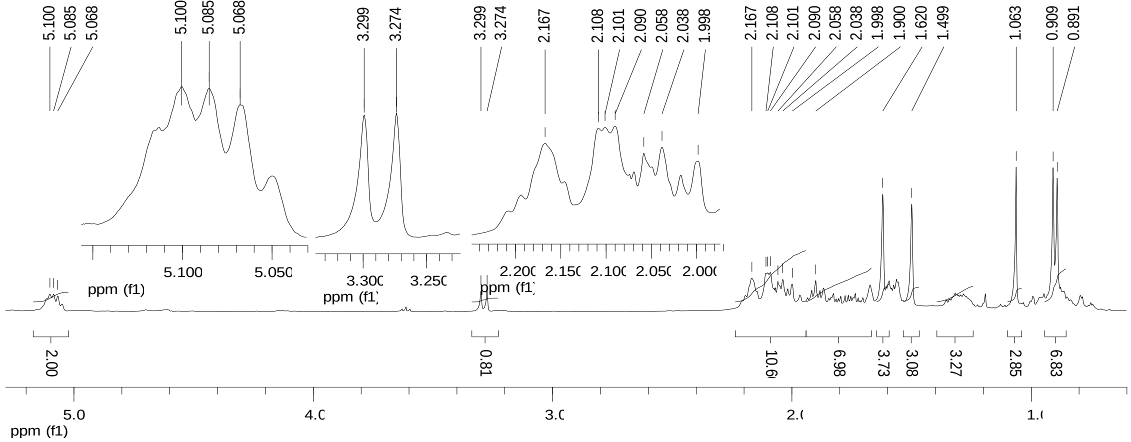
Appendix 11: NMR data of incensole acetate (**111**)

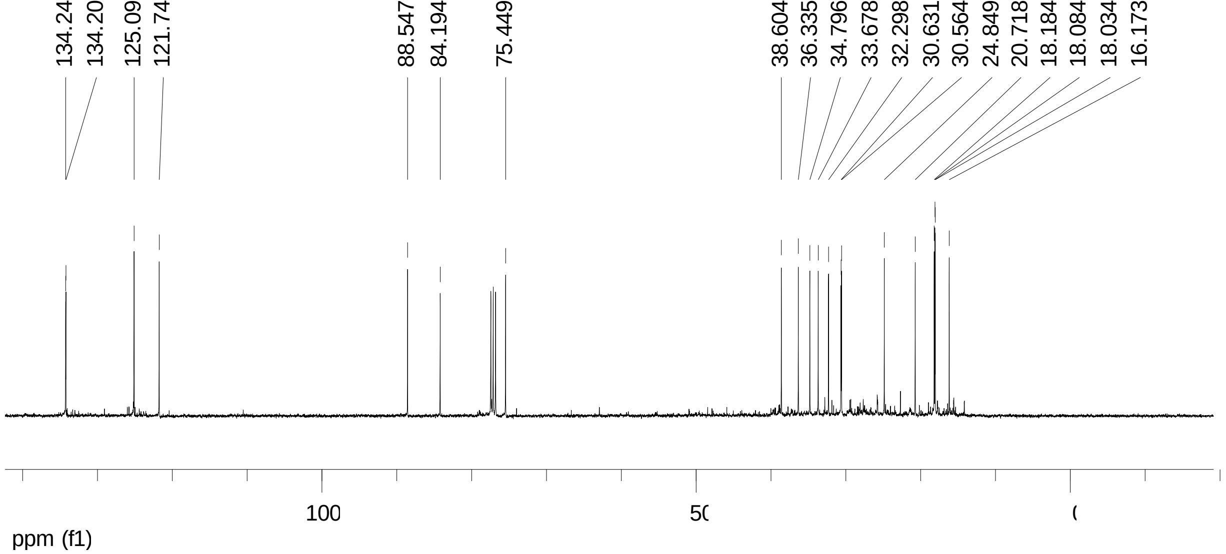

98-15B (InAc), 1H NMR, CDCl3

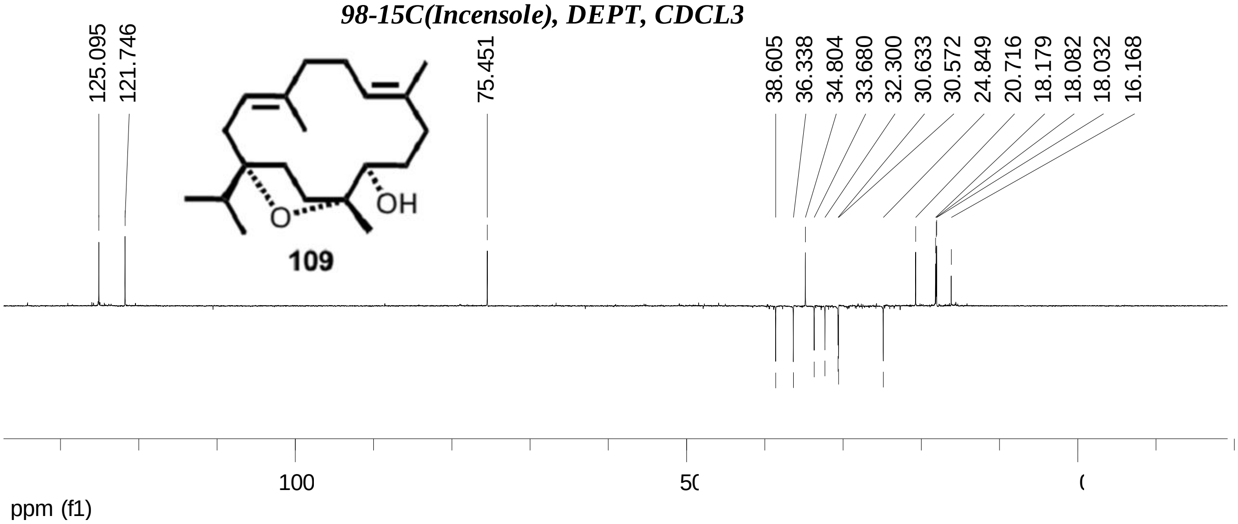

98-15 (BInAC), 13C NMR, CDCl3


98-15B (InAC), DEPT, CDCl3

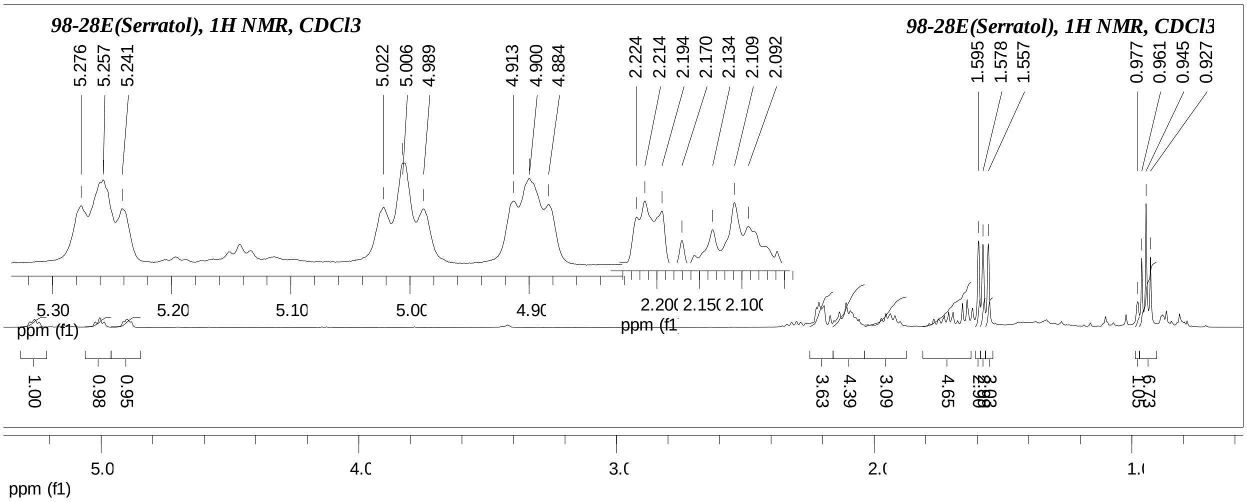
Appendix 12: NMR data of incensole (**5**)

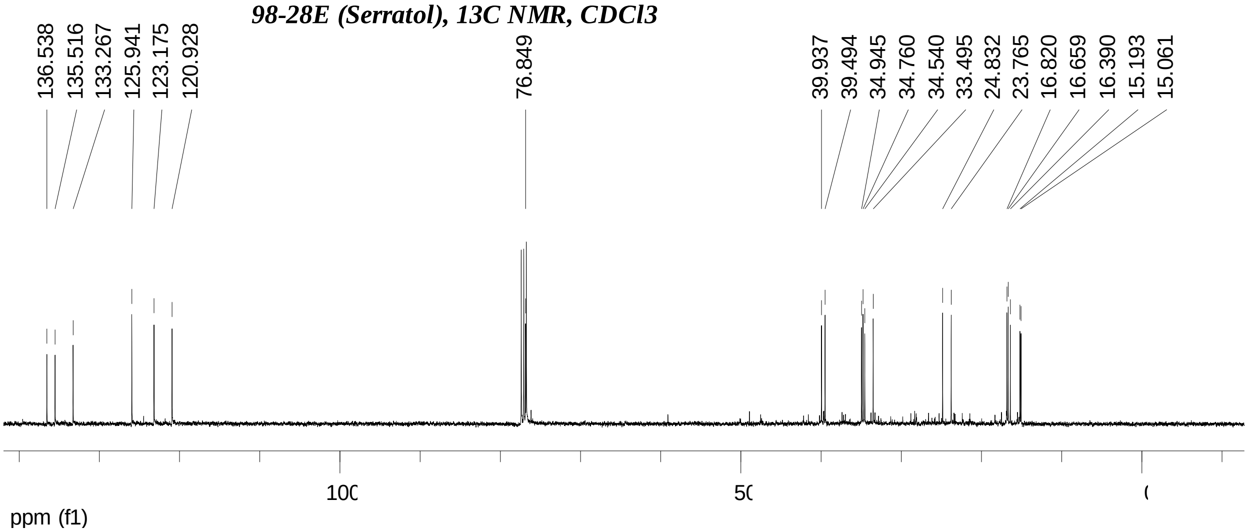

98-15C(Incensole), 1H NMR, CDCl3

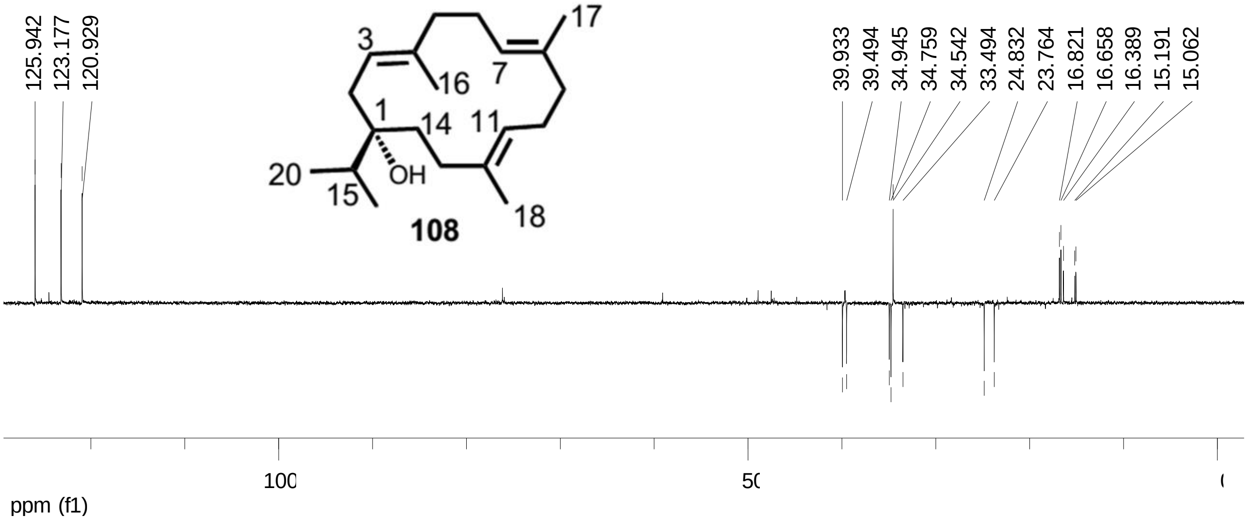

98-15C (Incensole), 13C NMR, CDCL3


98-15C(Incensole), DEPT, CDCL3

Appendix 13: NMR data of serratol (**108**)

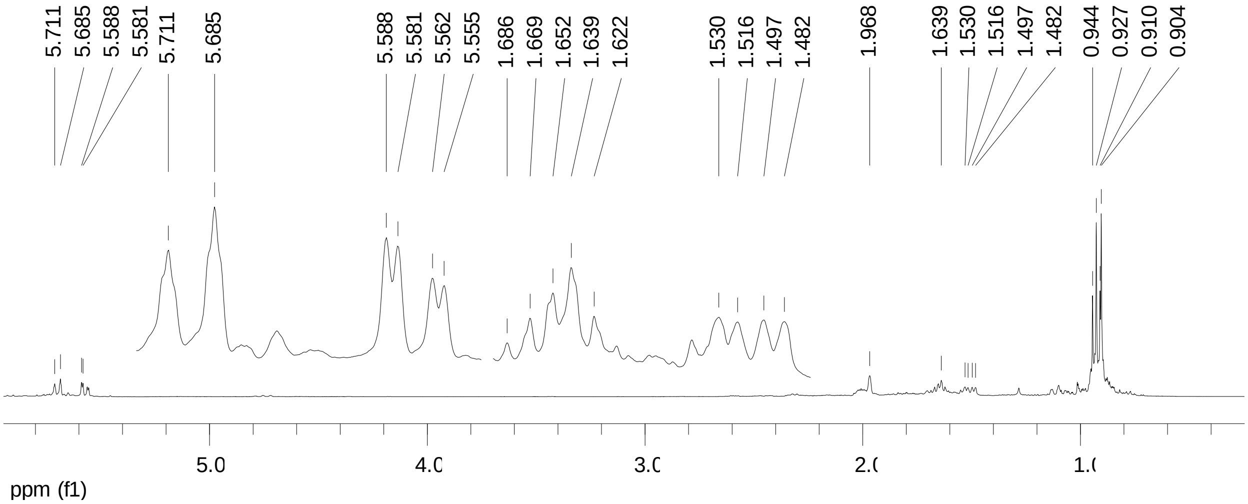




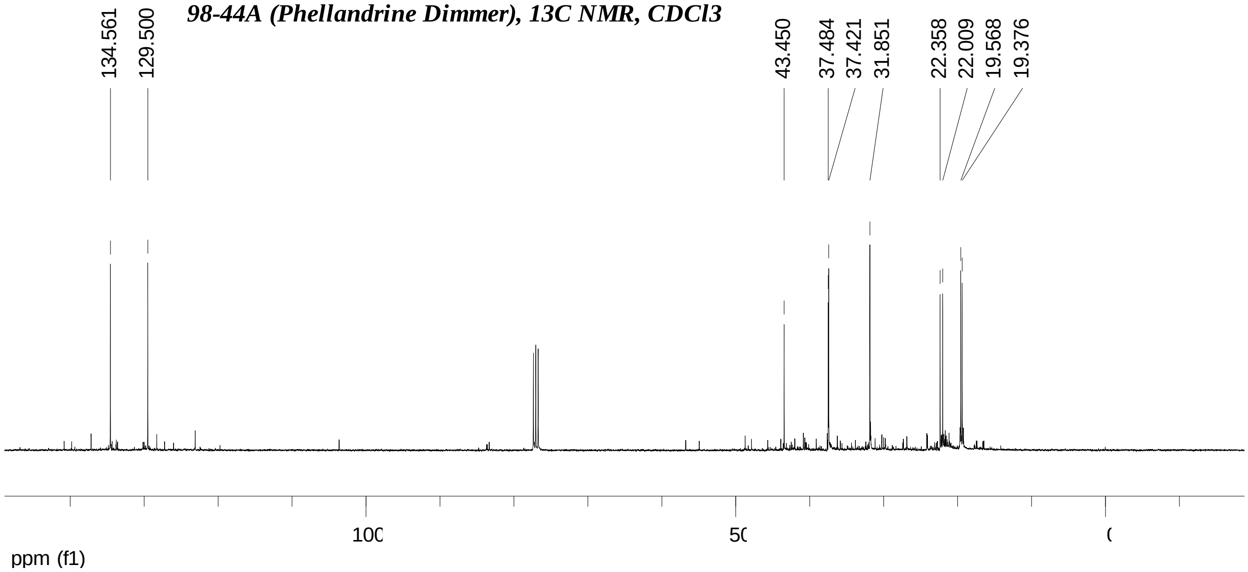


108

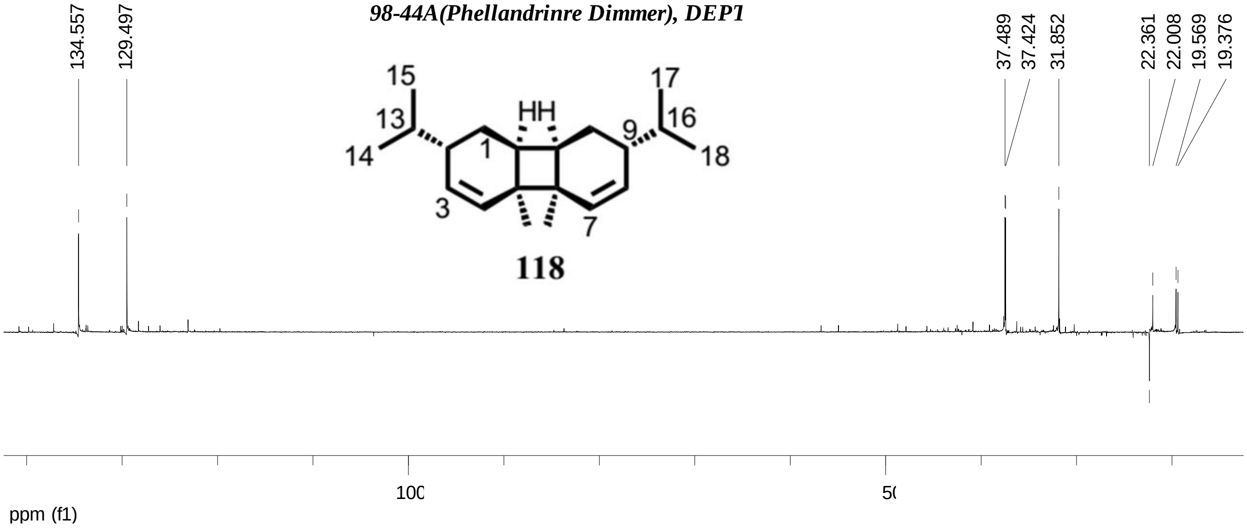
Appendix 14: NMR data of α-Phellandrene Dimer (**118**)



98-44A (Phellandrine dimmer), 1H NMR, CDCl3

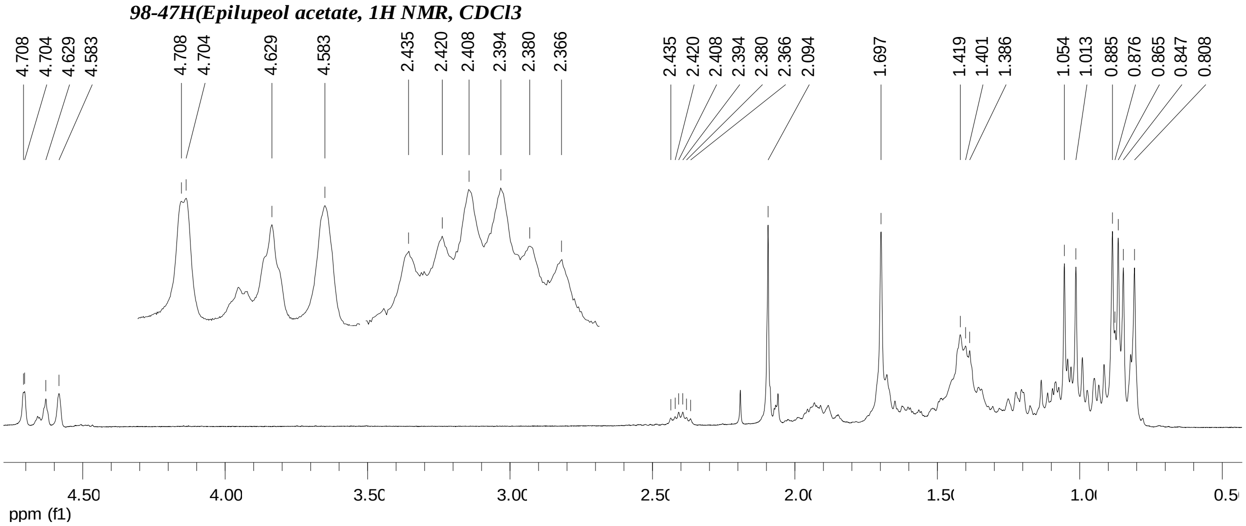


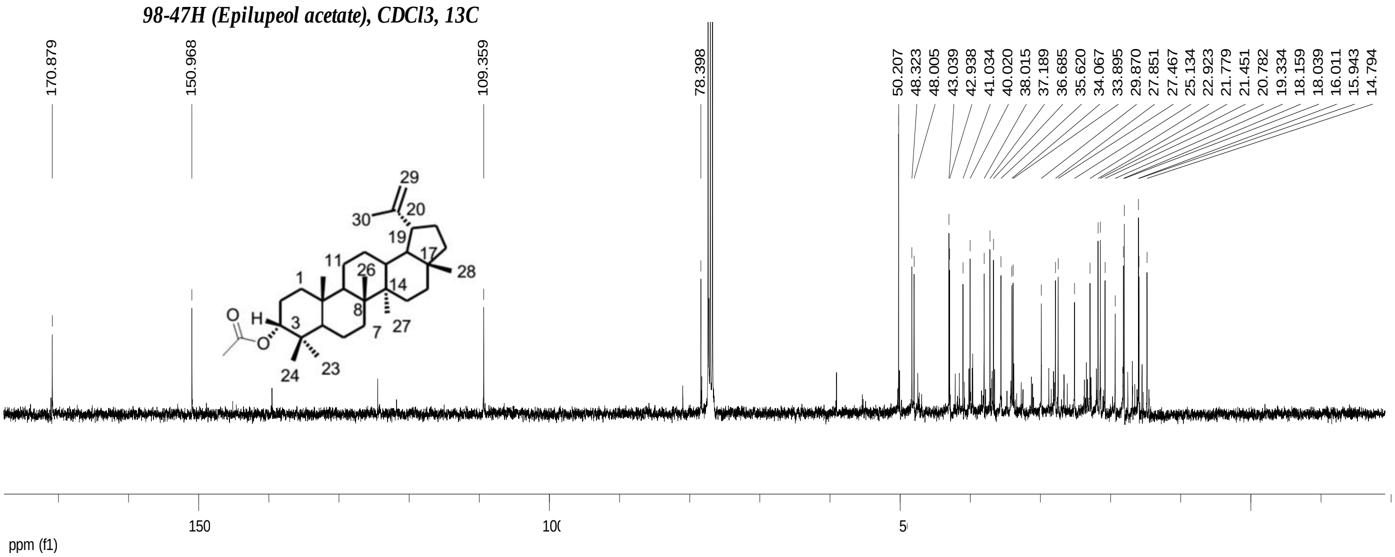
98-44A (Phellandrine Dimmer), 13C NMR, CDCl3

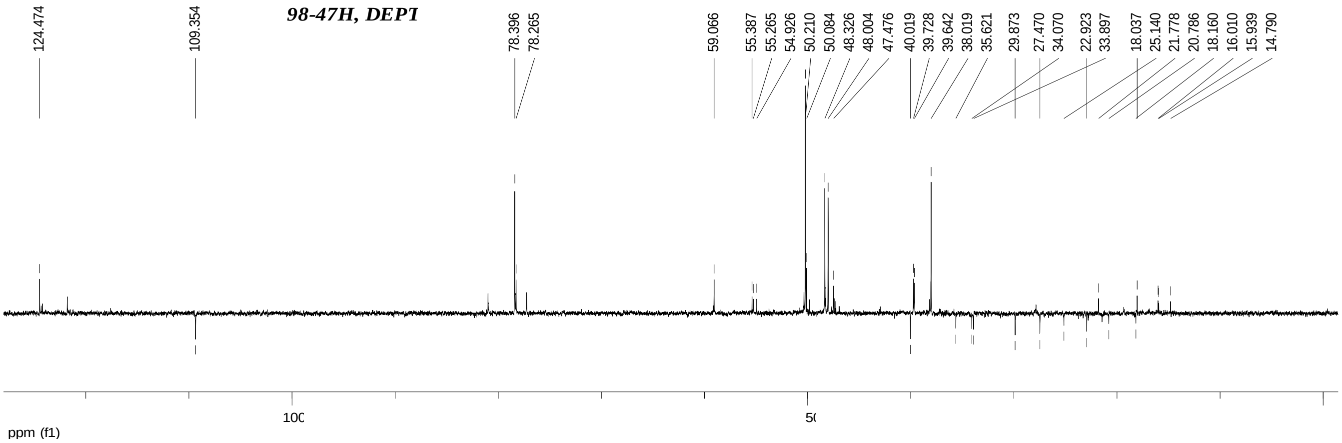


98-44A(Phellandrinre Dimmer), DEPT

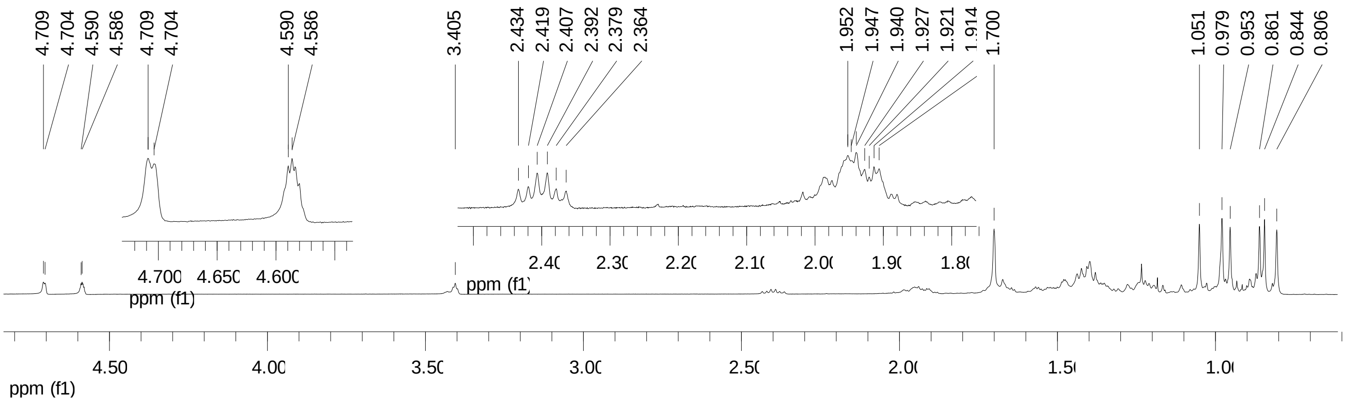
Appendix 15: NMR data of epilupeol acetate (**163**)

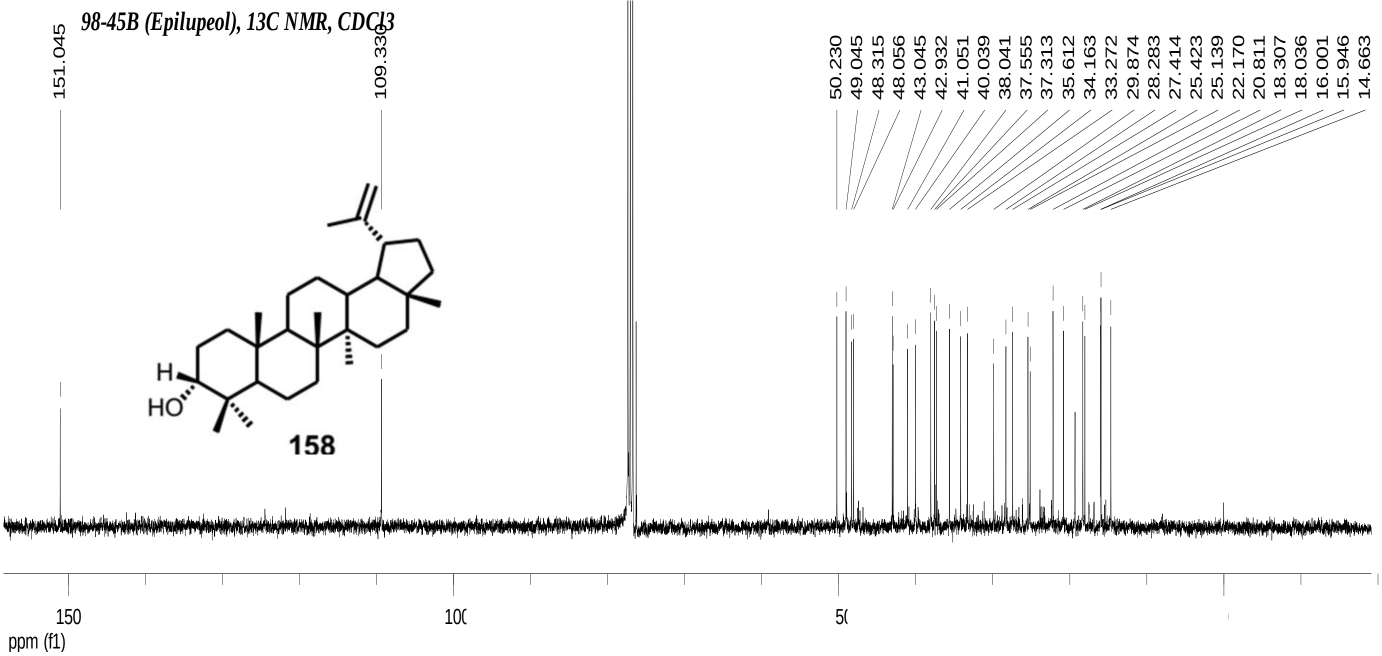




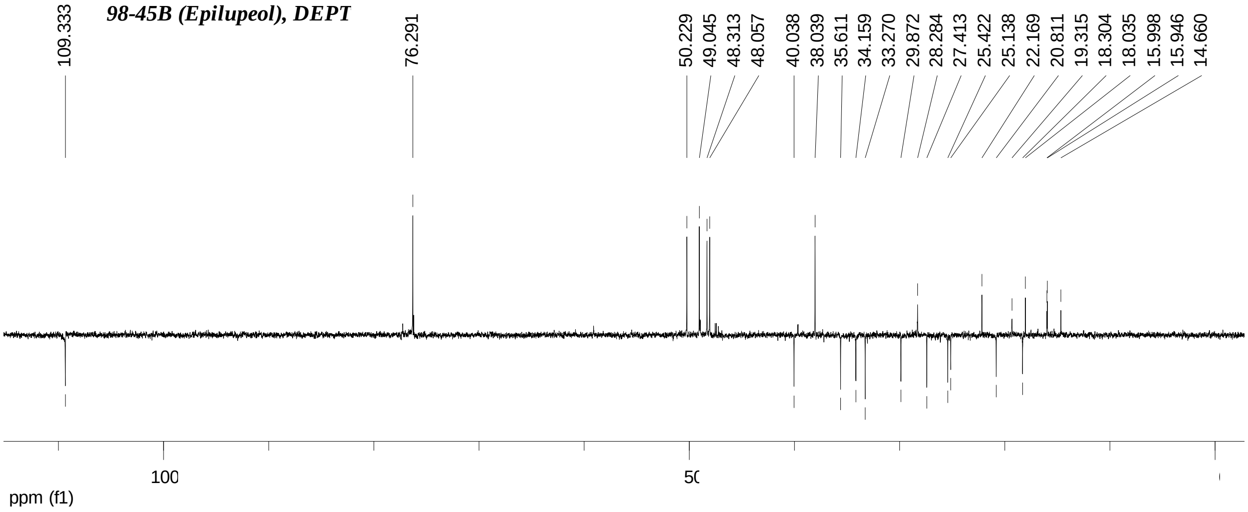


Appendix 16: NMR data of epilupeol (**158**)

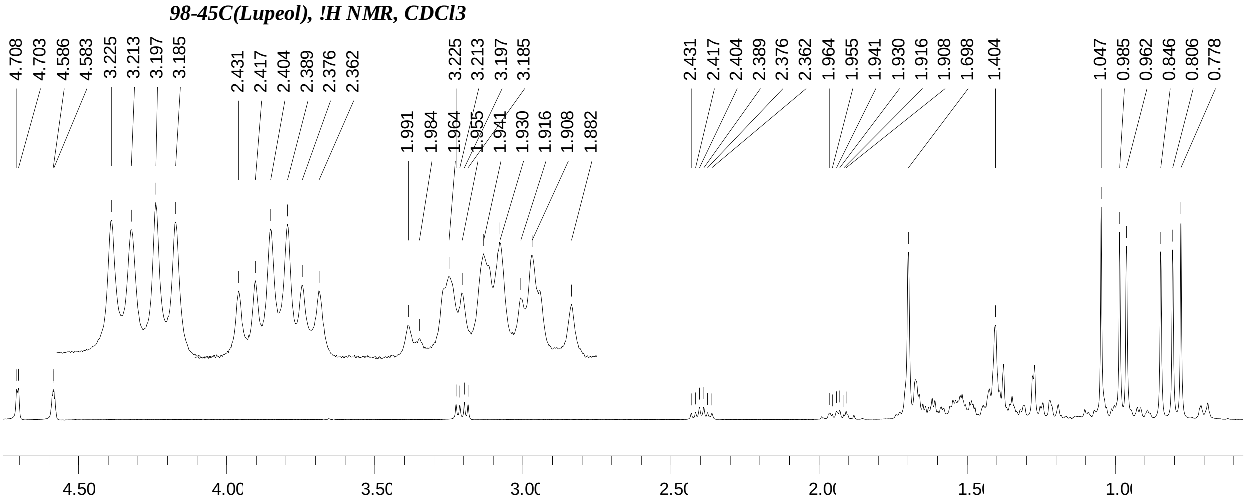

98-45B (Epilupeol), 1HNMR, CDCl3

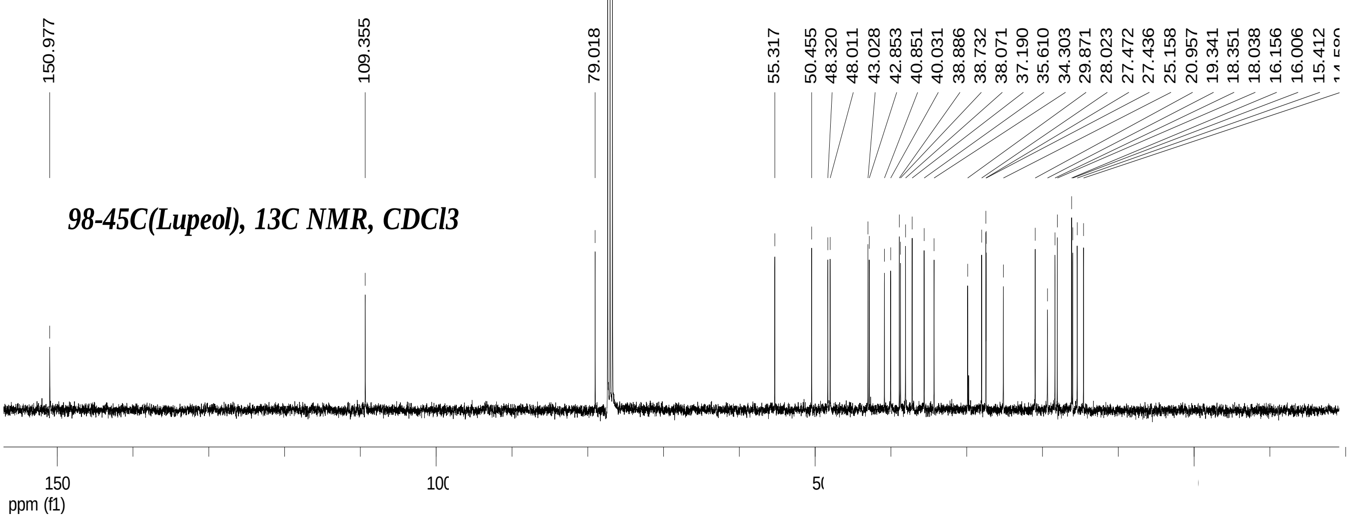

98-45B (Epilupeol), 13C NMR, CDCl3

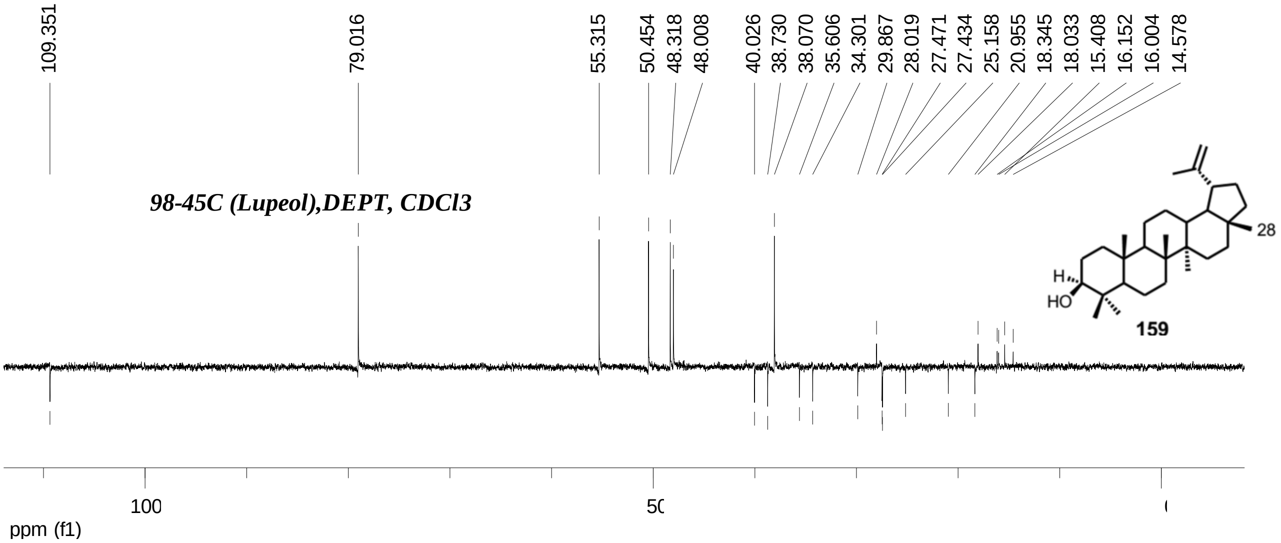
158


98-45B (Epilupeol), DEPT

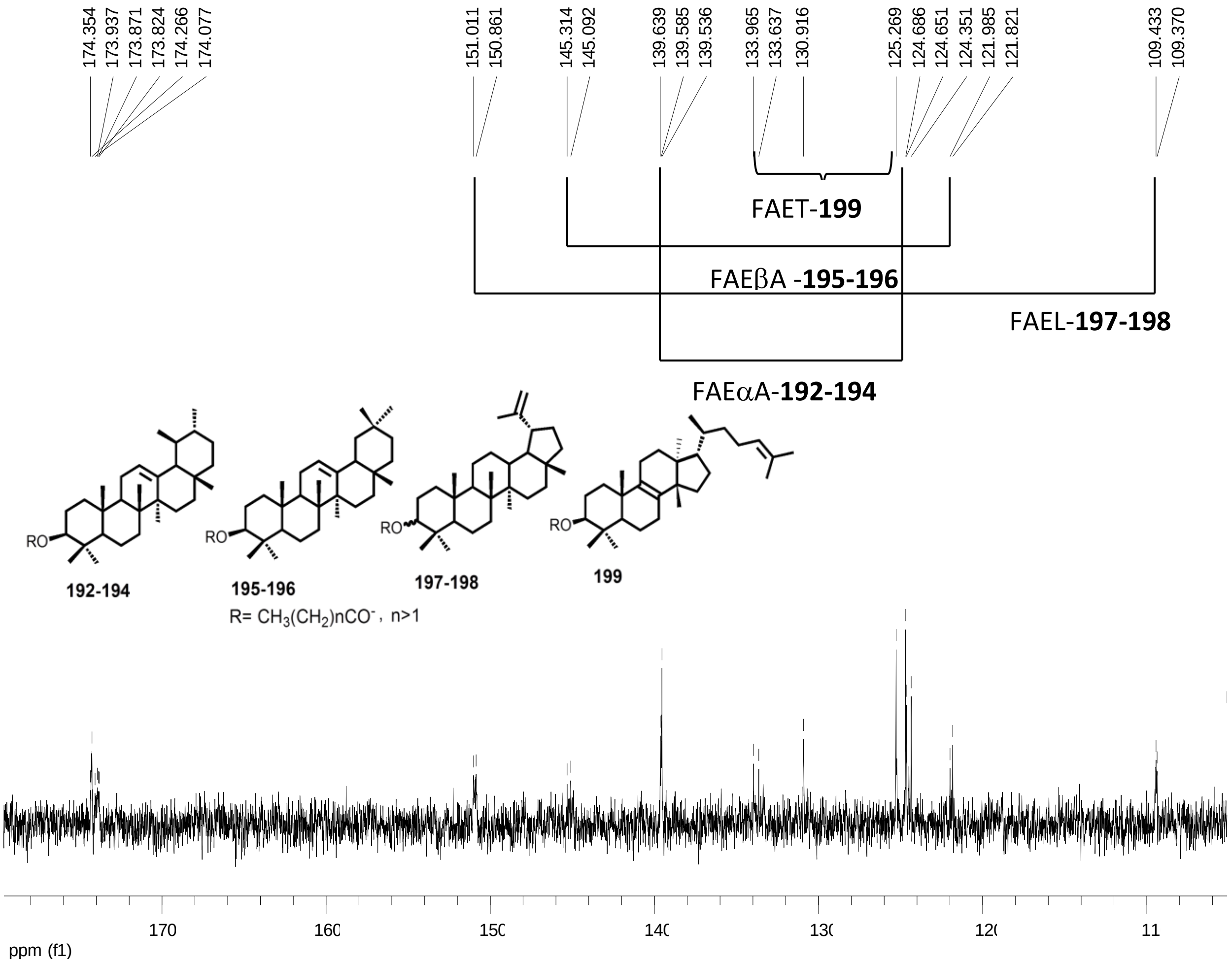
Appendix 17: NMR data of lupeol (**159**)



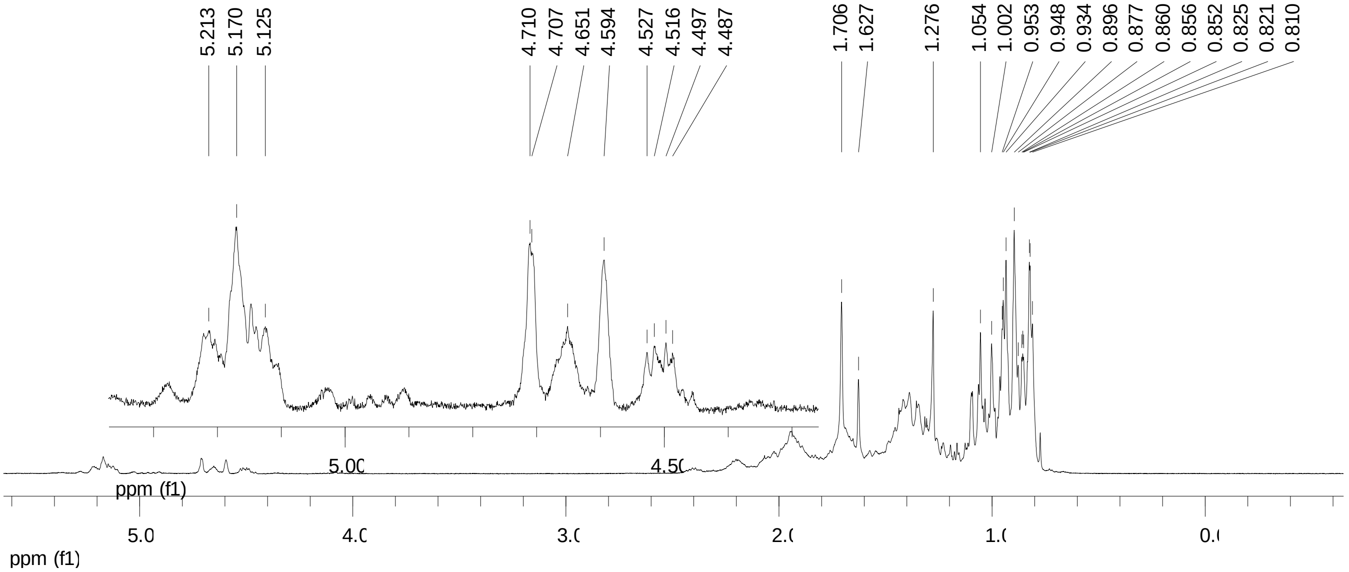




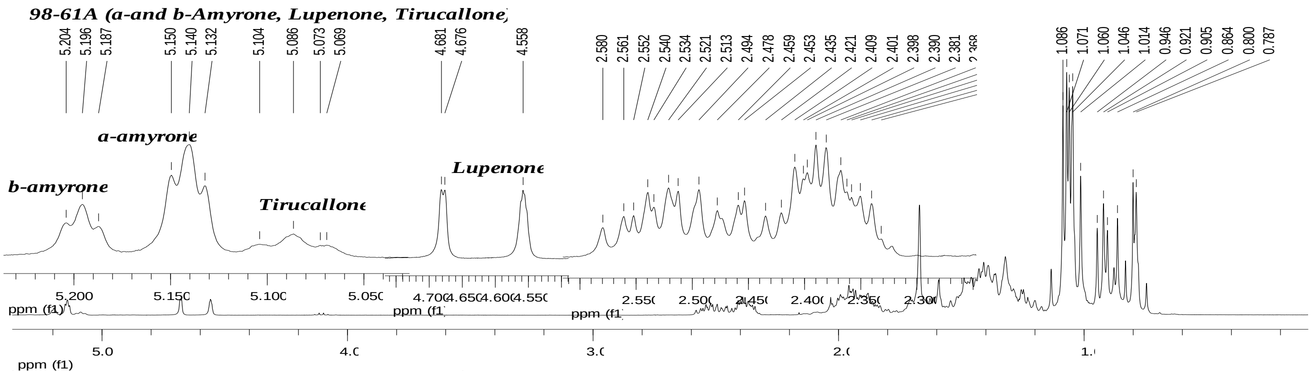
Appendix 18: NMR data of fatty acid esters of eight Triterpenes

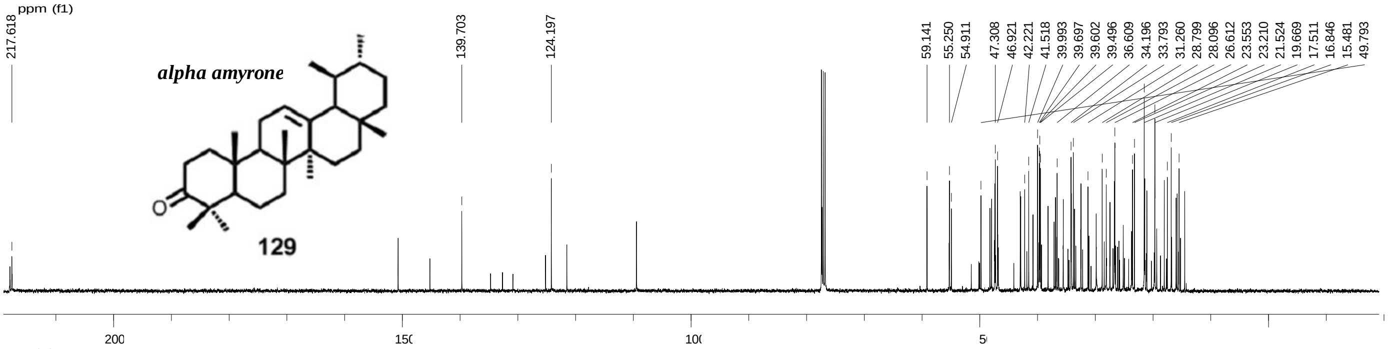


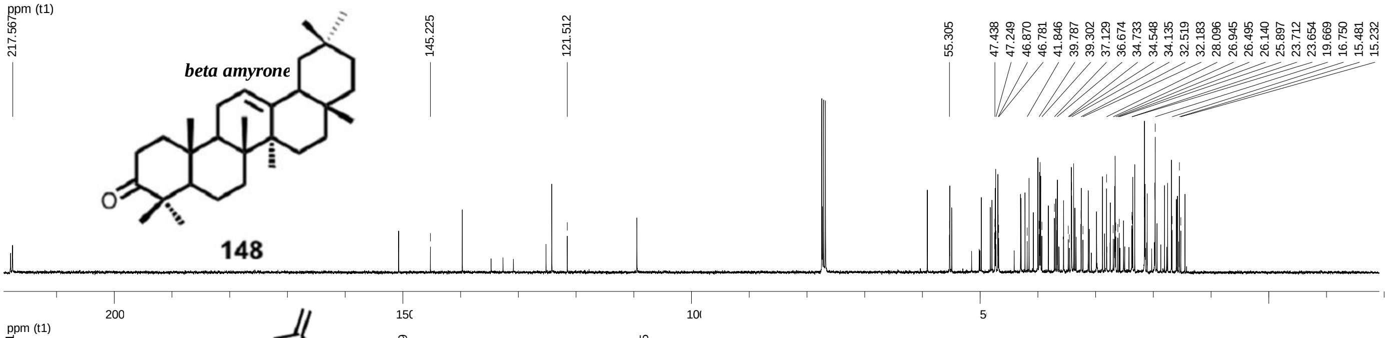
98-61D (FAE of eight triterpenes), 1H NMR, CDCl3

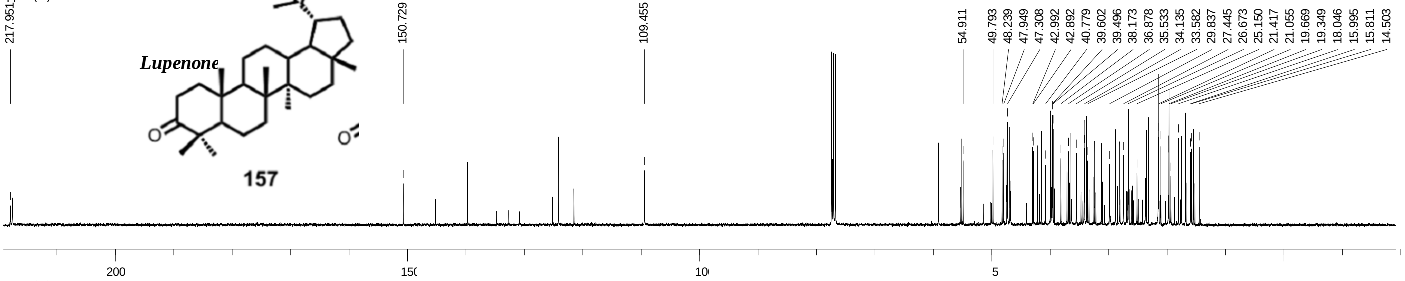


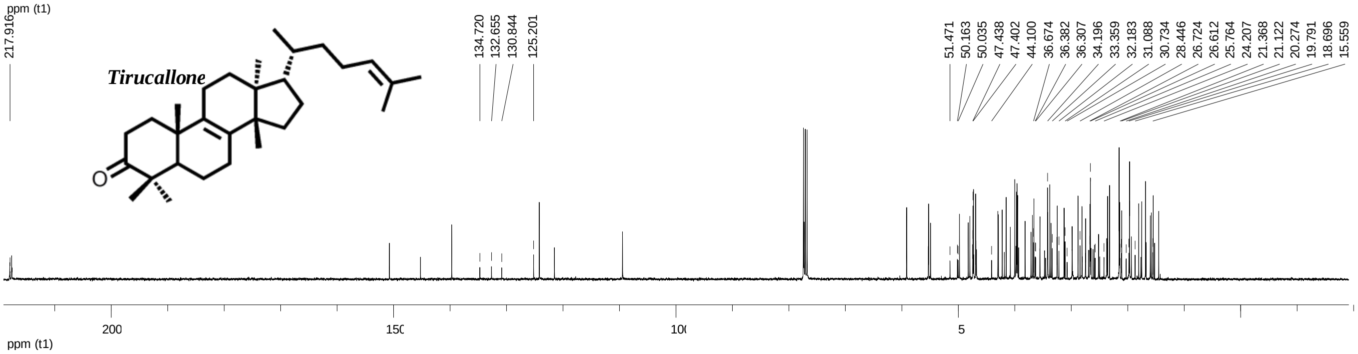
Appendix 19: α(**129**)-and β-amyrone (**148**), lupenone (**157**), tirucallone (**170**))



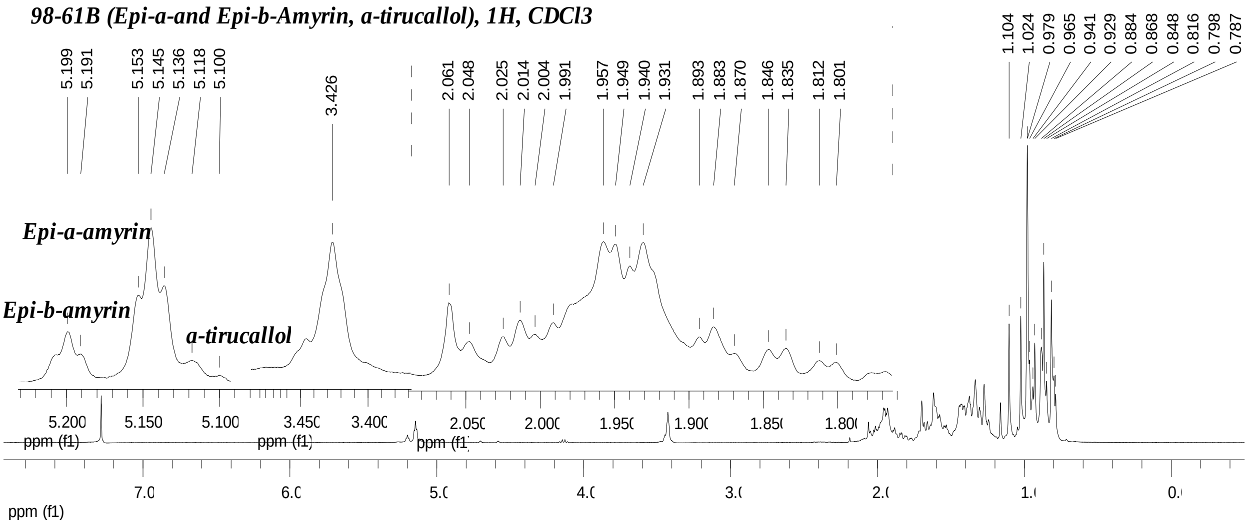


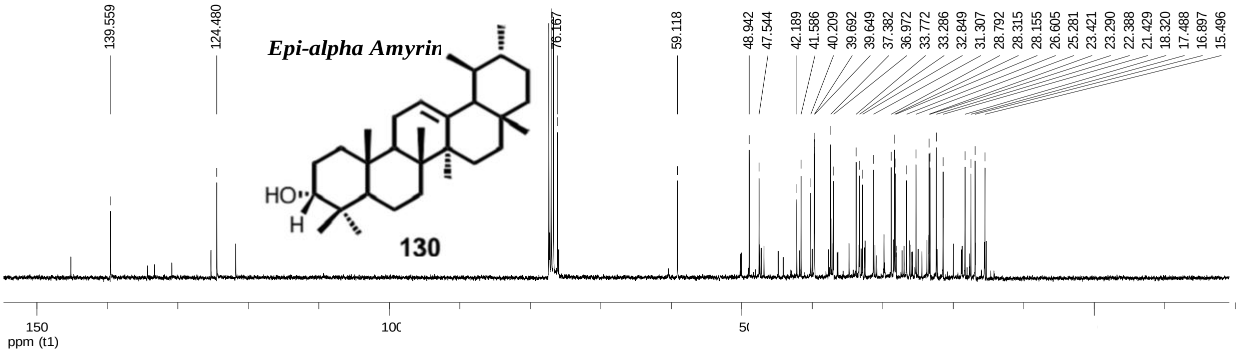


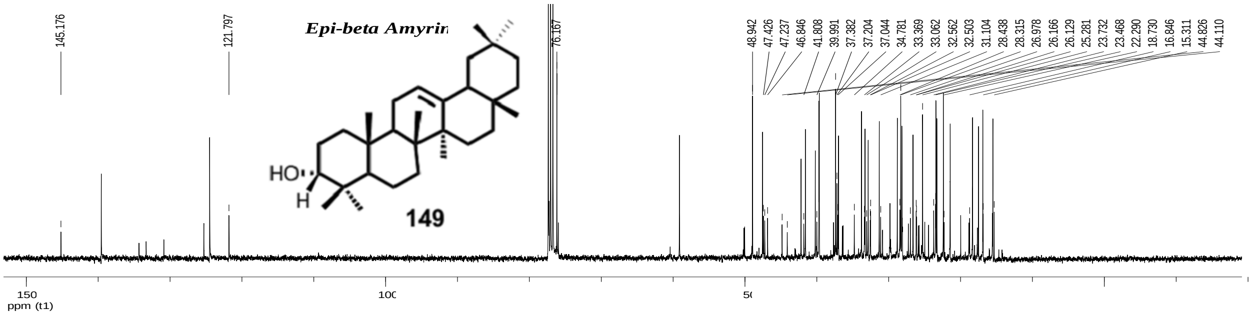


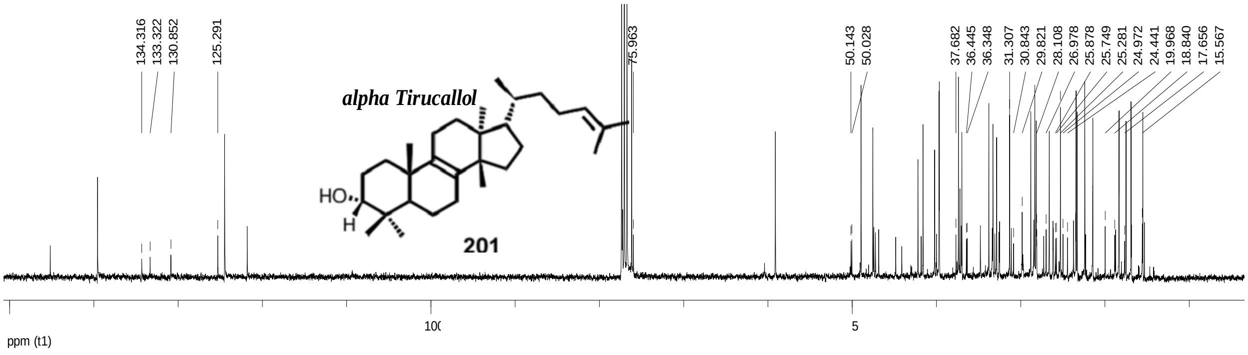


Appendix 20: NMR data of e*pi*-α- (**130**) and e*pi*-β-Amyrin (**149**), α-tirucallol(**201**)

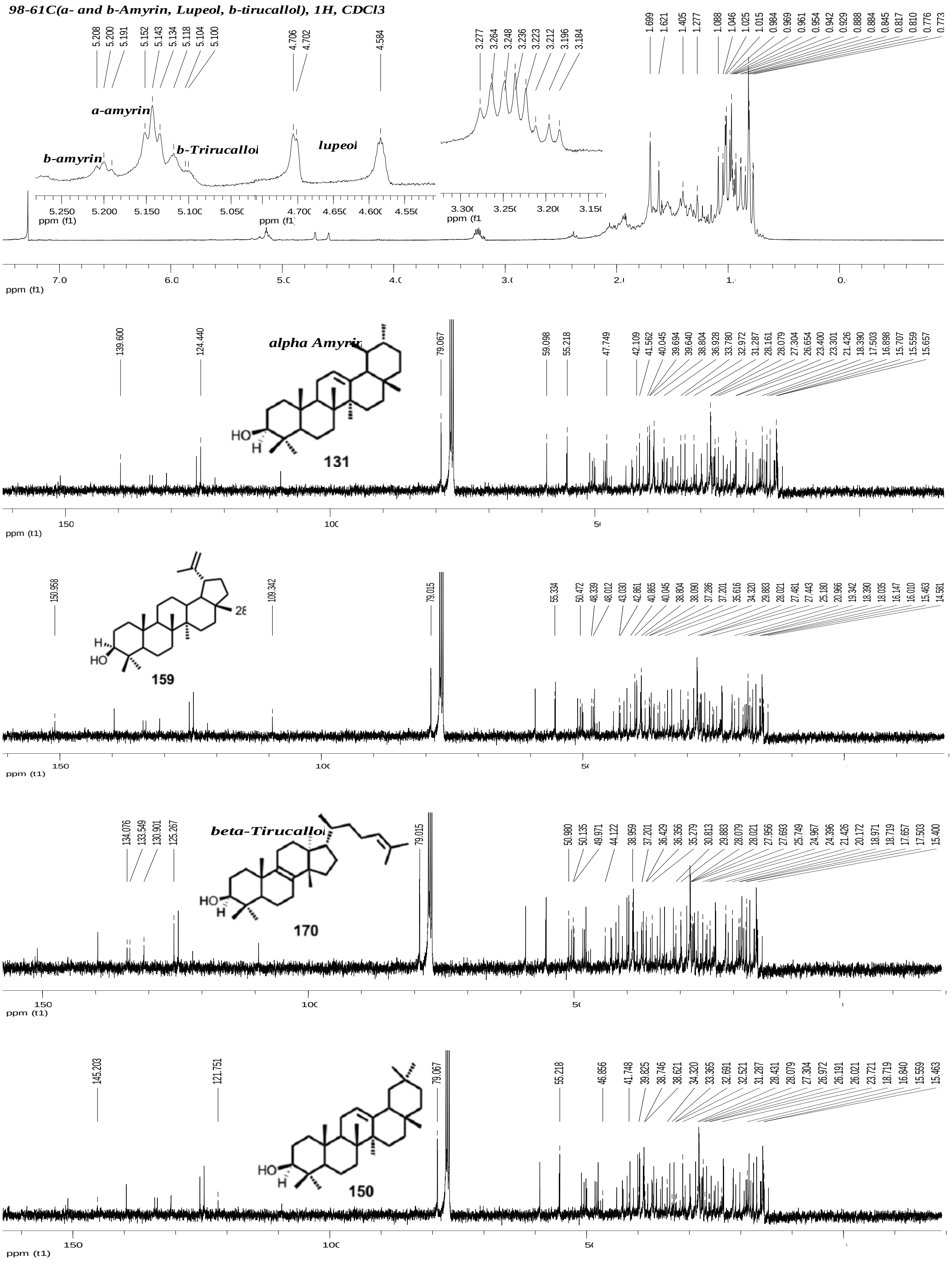




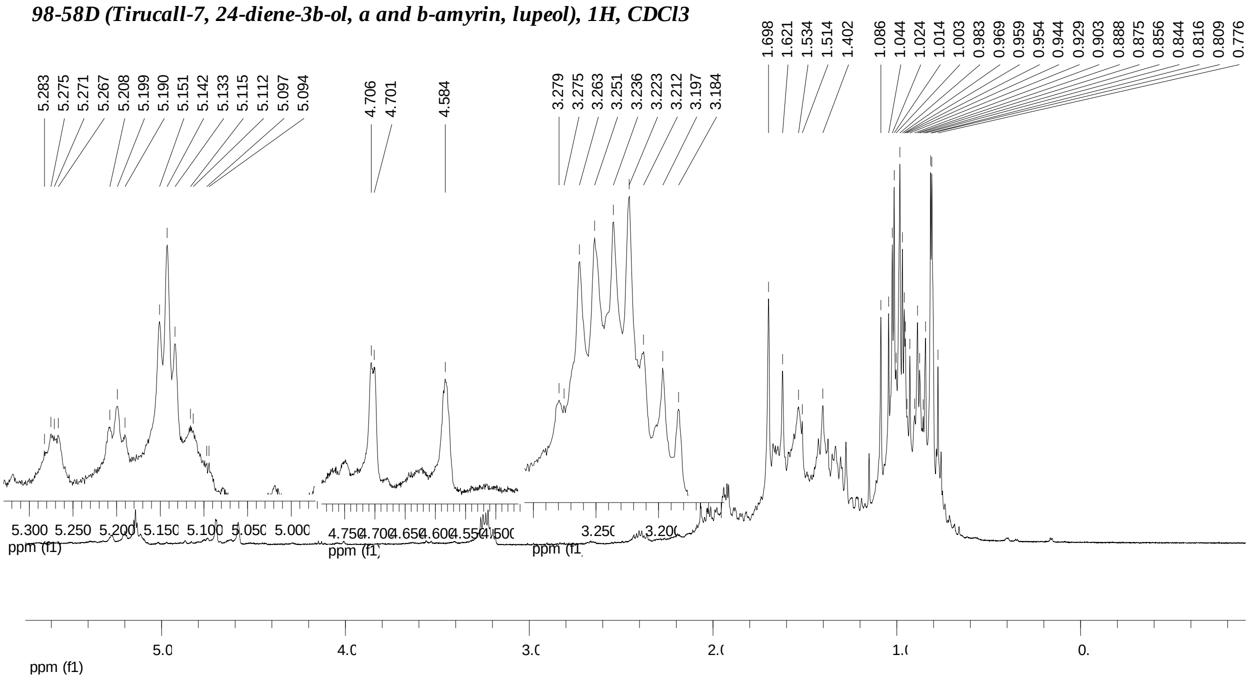


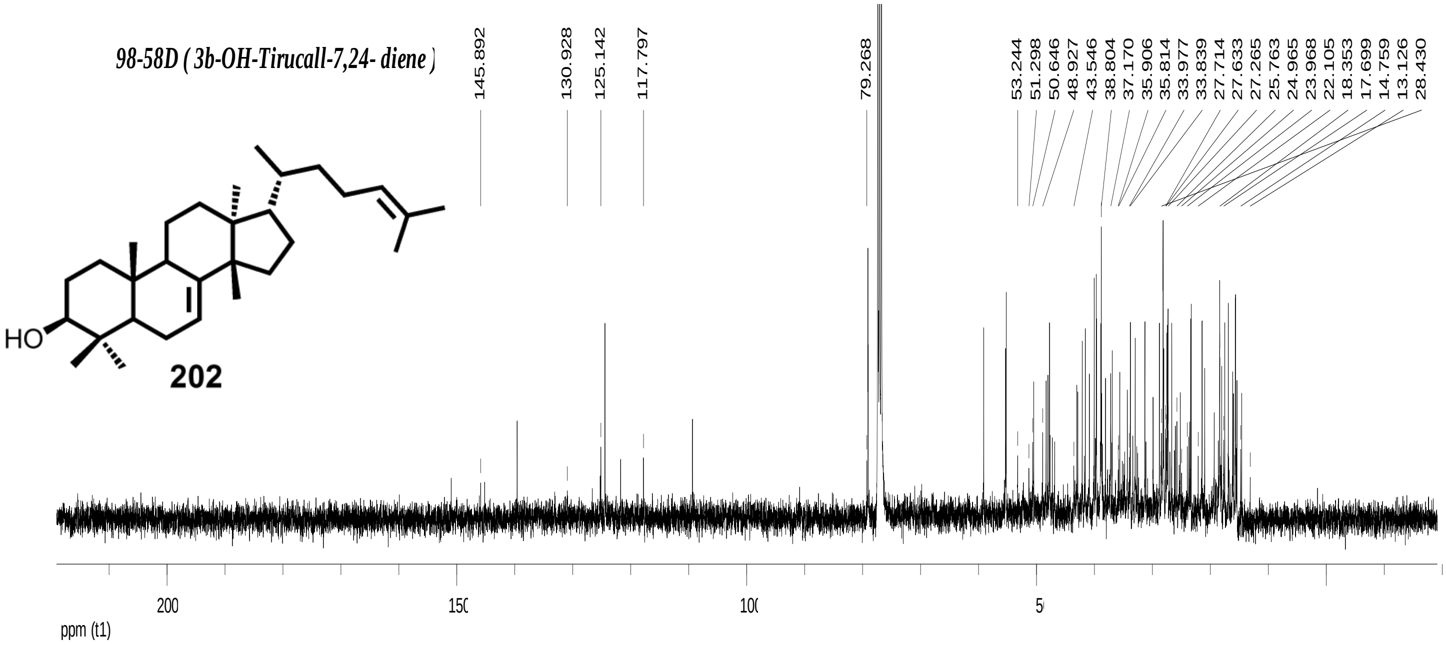


Appendix 21: NMR data of α (**131**)-and β-amyrin (**150**), lupeol (**159**), β-tirucallol (**170**)



Appendix 22: NMR data of tirucall-7,24-diene-β-ol (**202**), α and β-amyrin, lupeol

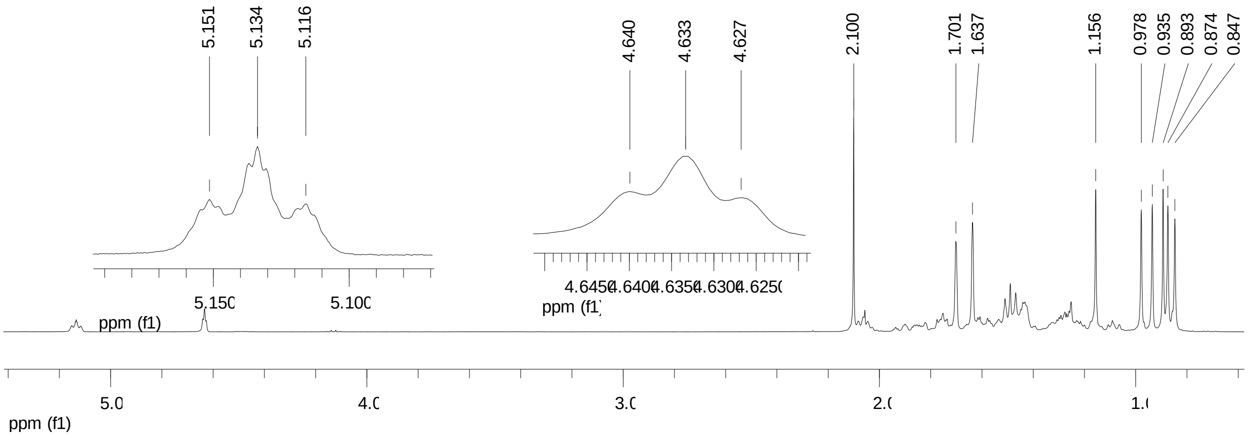




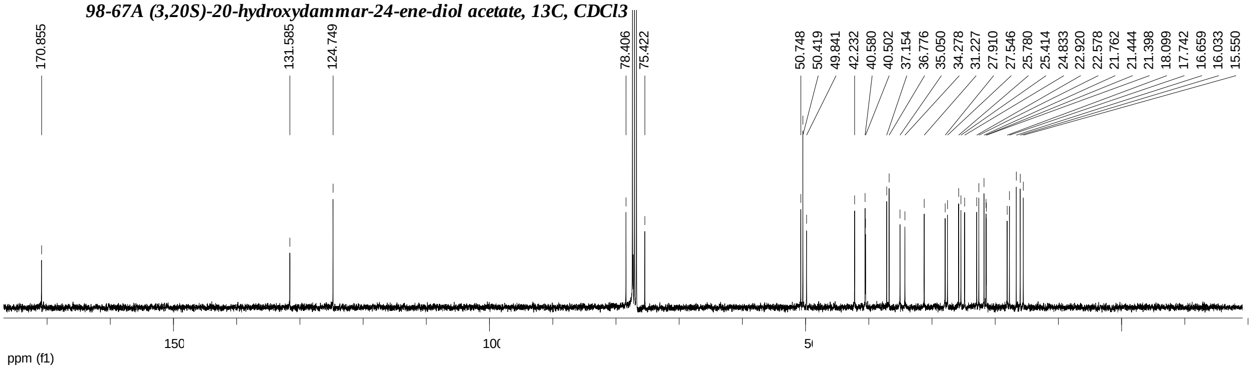
202

Appendix 23: NMR data of 20-hydroxydammar-24-ene-diol acetate (**203**)

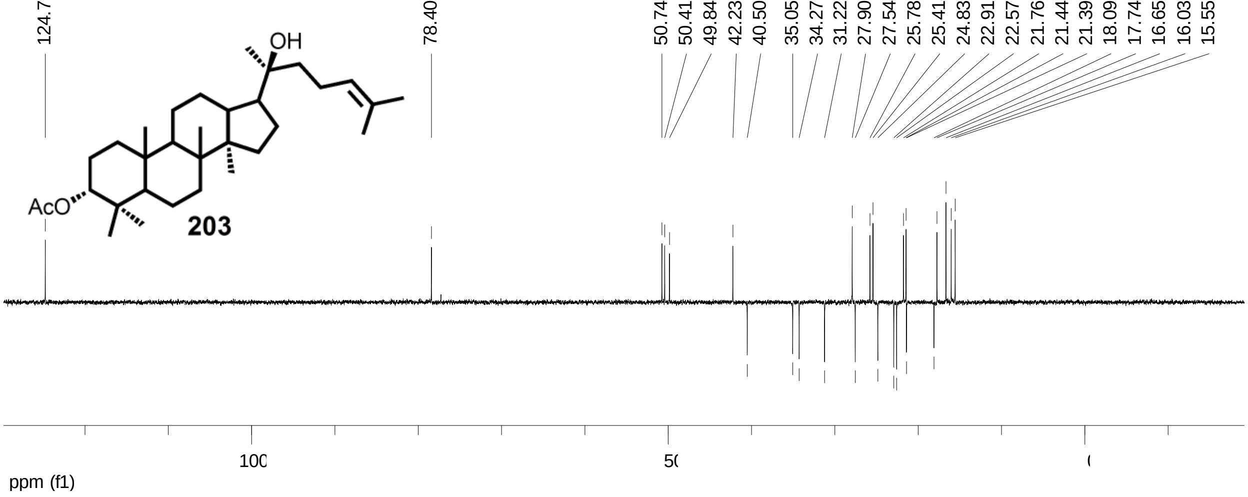
98-67A (3,20S)-20-hydroxydammar-24-ene-diol acetate, 1H, CDCl3



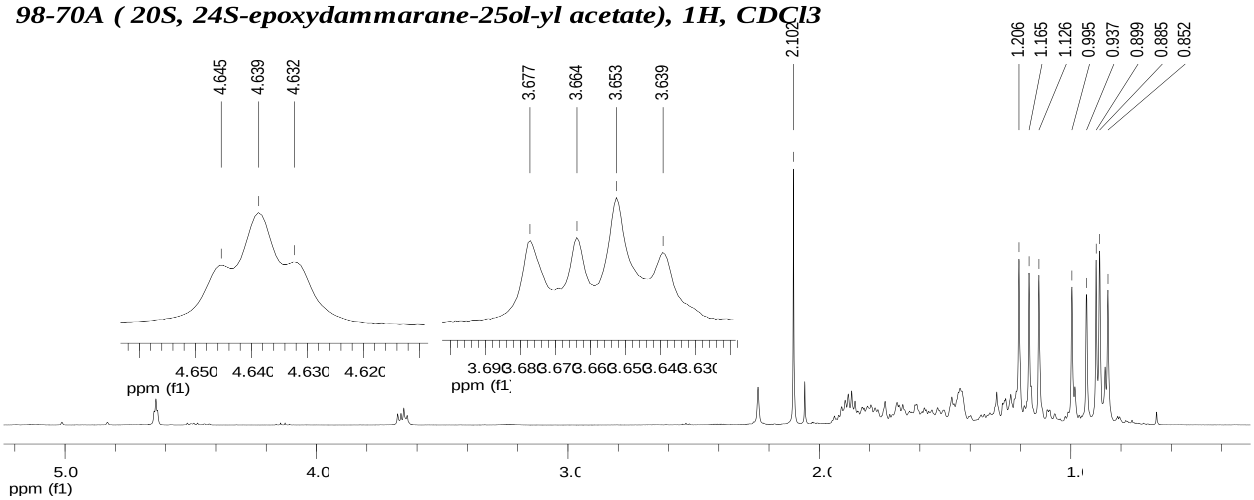
98-67A (3,20S)-20-hydroxydammar-24-ene-diol acetate, 13C, CDCl3

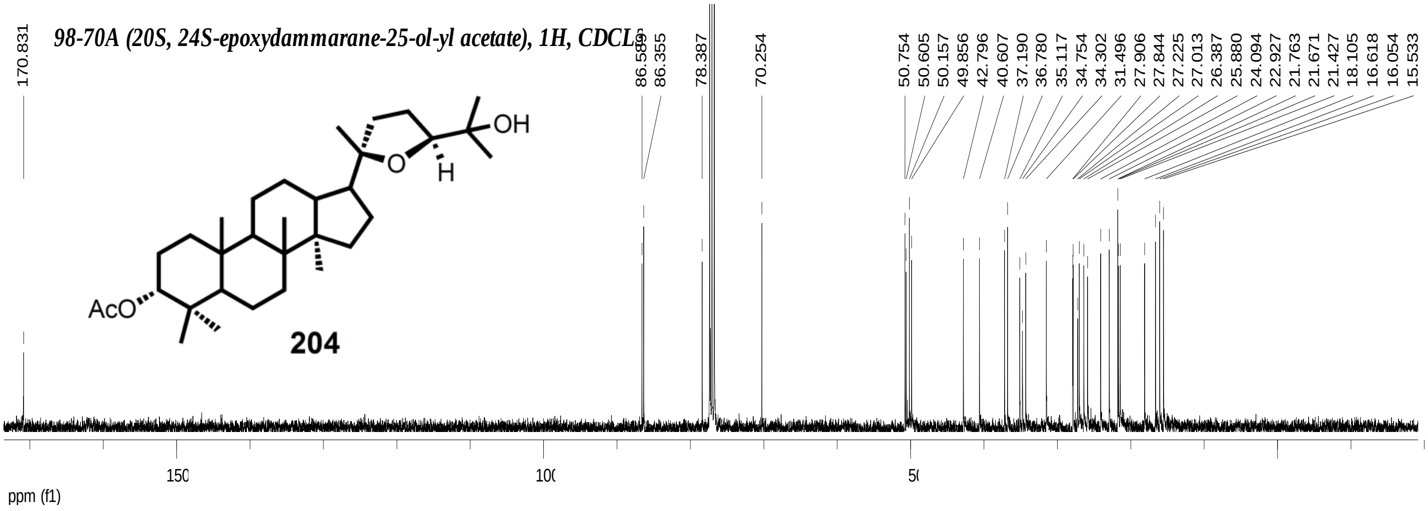


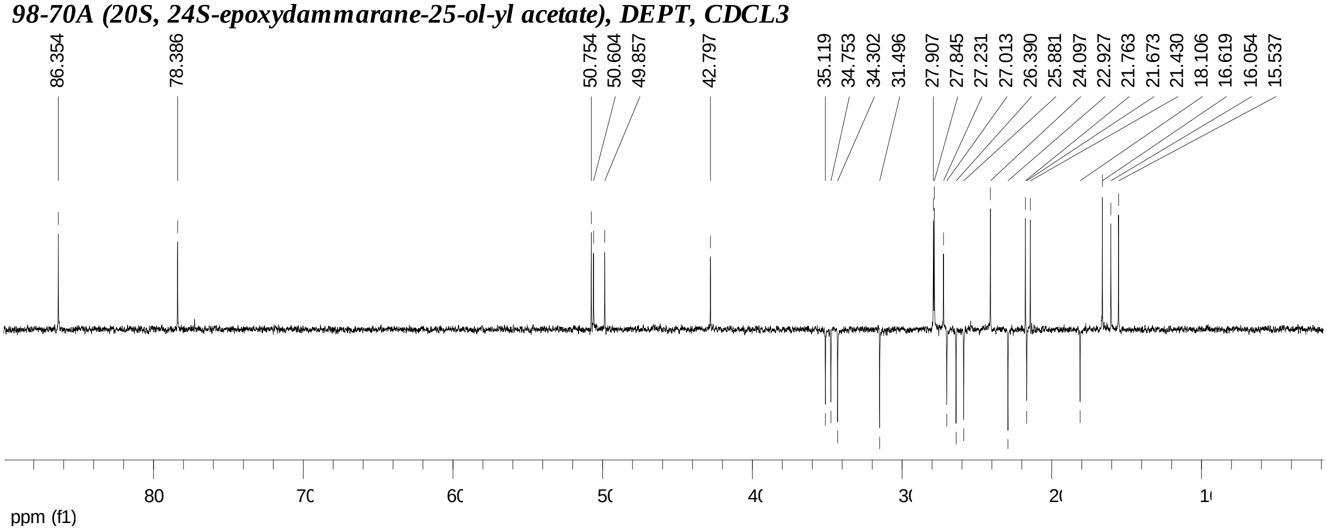
98-67A (3,20S)-20-hydroxydammar-24-ene-diol acetate, DEPT, CDCl3



Appendix 24: NMR data of 20S,24S-epoxydammarane-25-ol-yl acetate (**204**)

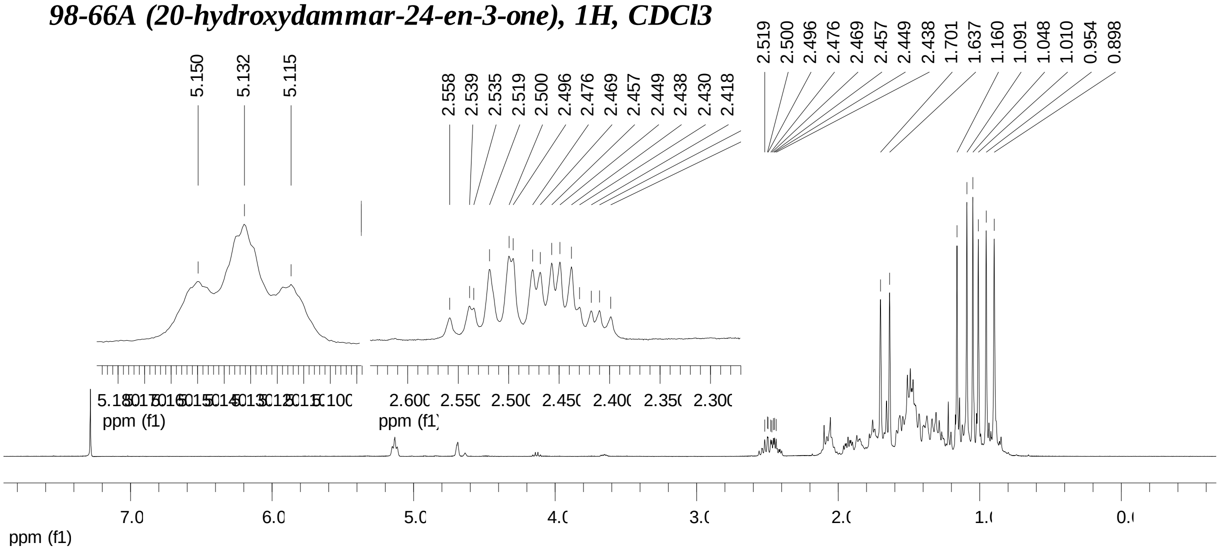

98-70A (20S, 24S-epoxydammarane-25ol-yl acetate), 1H, CDCl3


98-70A (20S, 24S-epoxydammarane-25-ol-yl acetate), 1H, CDCL3

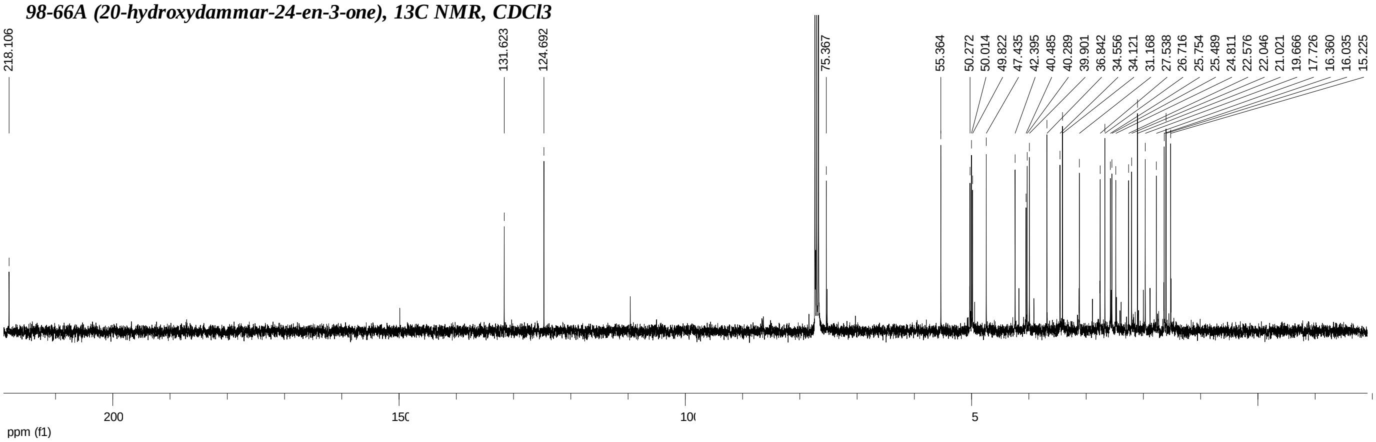

98-70A (20S, 24S-epoxydammarane-25-ol-yl acetate), DEPT, CDCL3

Appendix 25: NMR data of 20-hydroxydammar-24-en-3-one (**205**)

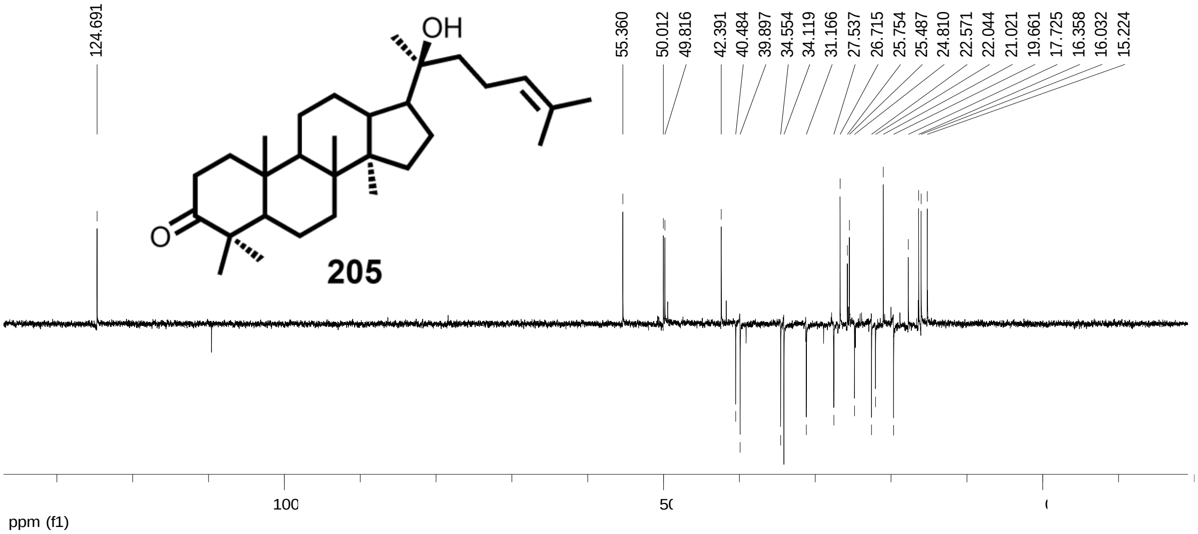
98-66A (20-hydroxydammar-24-en-3-one), 1H, CDCl3



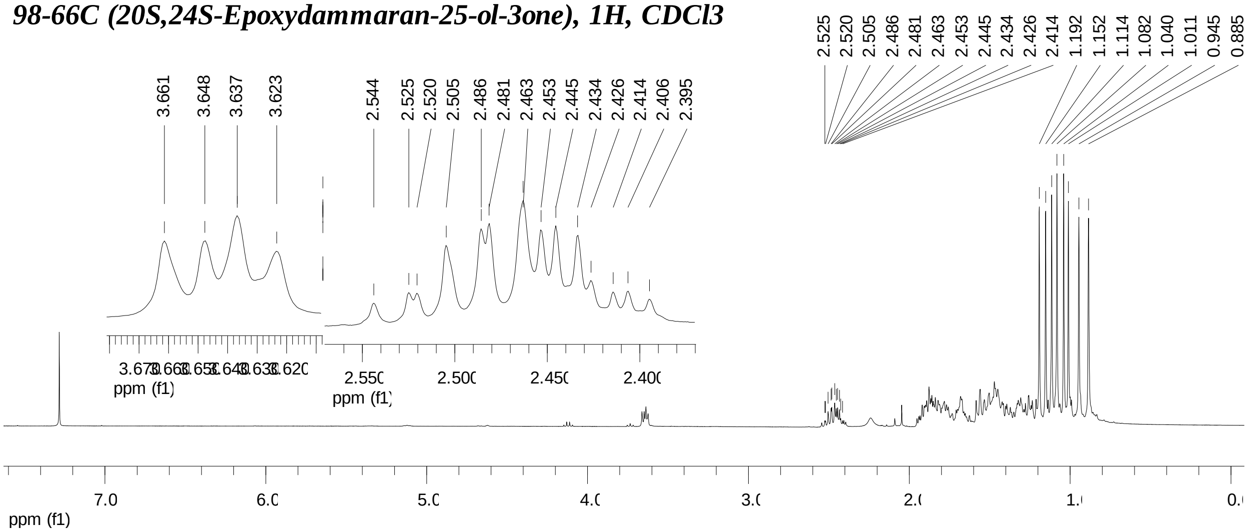
98-66A (20-hydroxydammar-24-en-3-one), 13C NMR, CDCl3

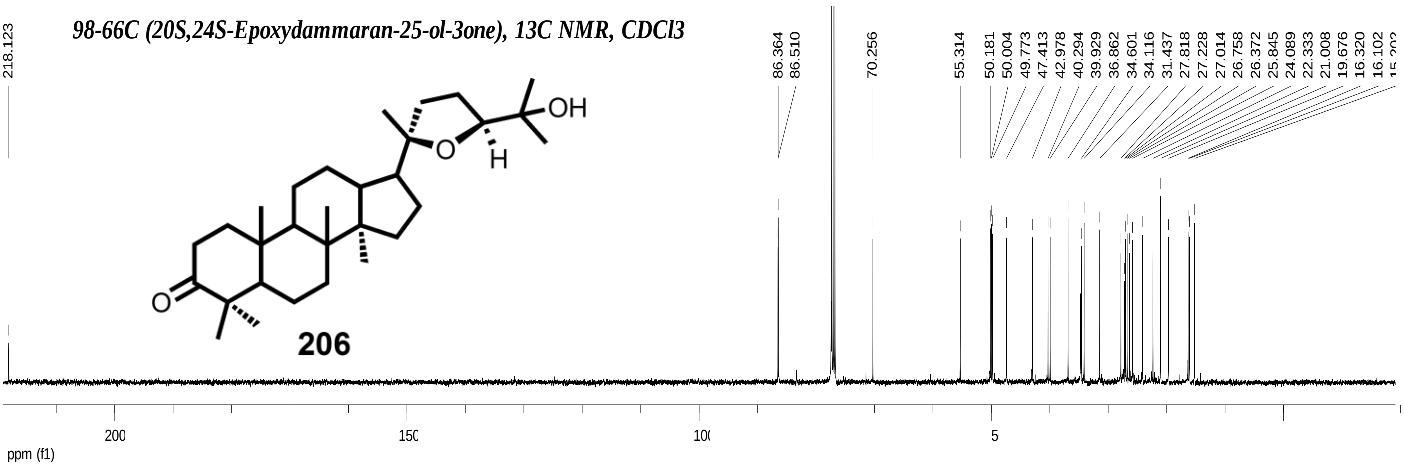


98-66A (20-hydroxydammar-24-en-3-one), DEPT, CDCl3

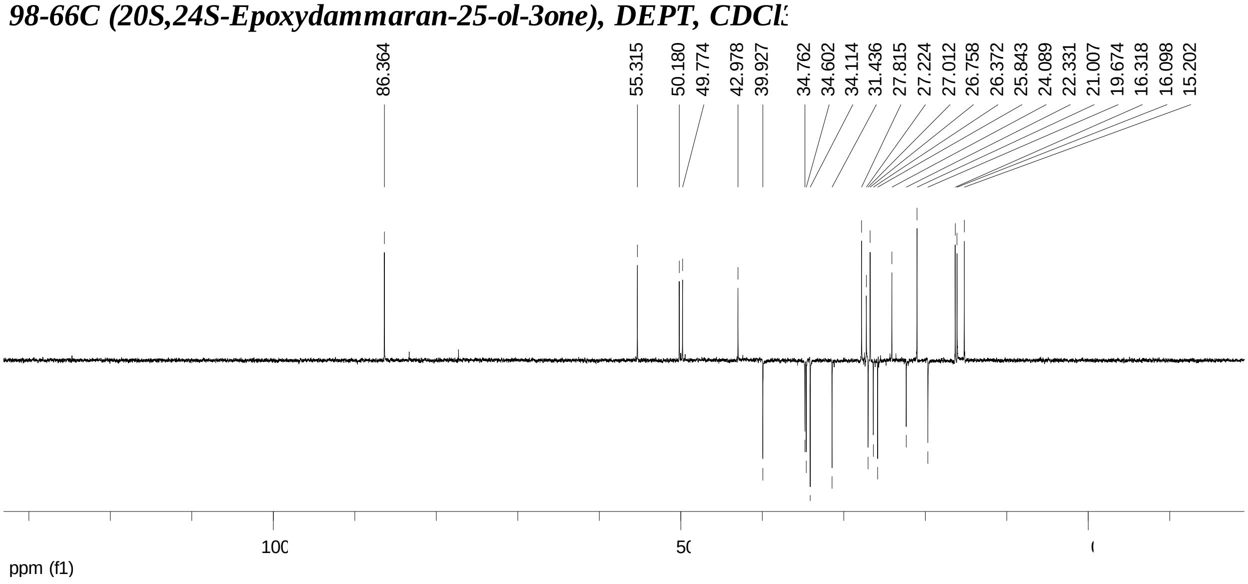


Appendix 26: NMR data of 20S,24S-epoxydammaran-25-ol-3one (**206**)


98-66C (20S,24S-Epoxydammaran-25-ol-3one), 1H, CDCl3

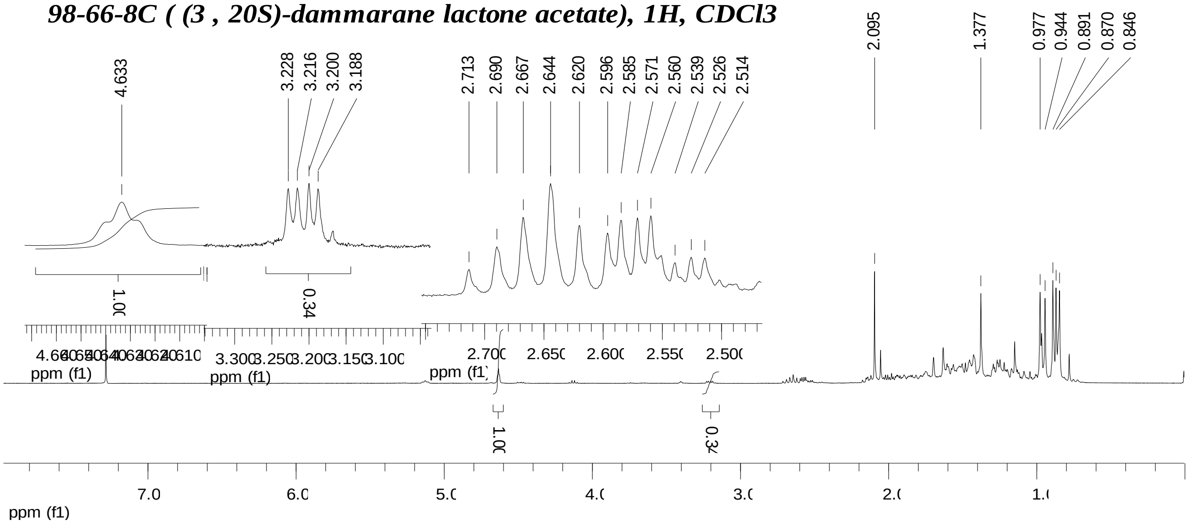

98-66C (20S,24S-Epoxydammaran-25-ol-3one), 13C NMR, CDCl3

206

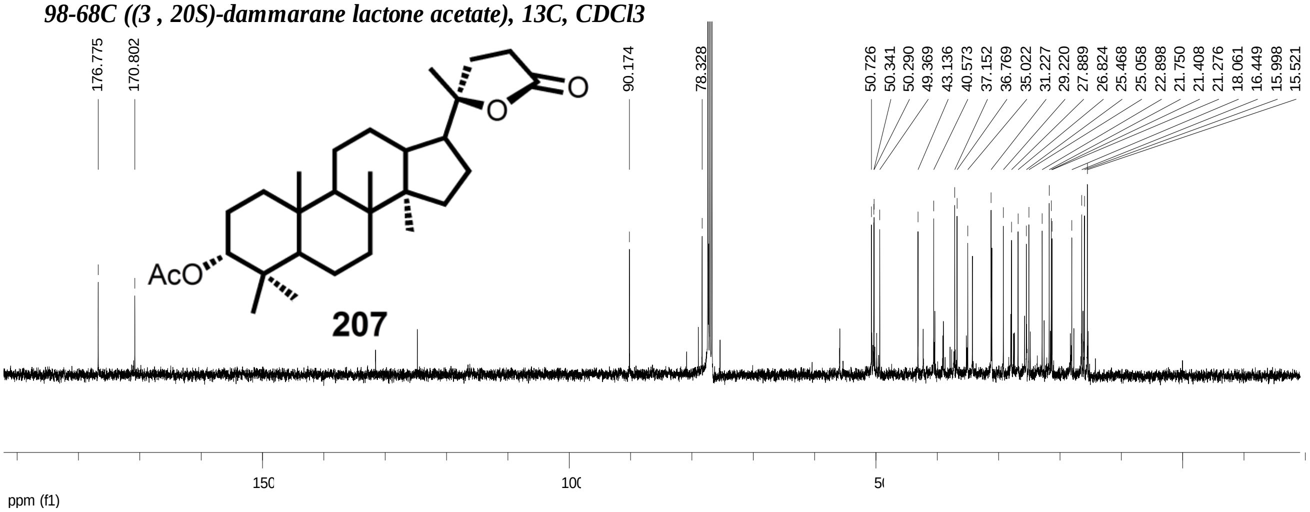

98-66C (20S,24S-Epoxydammaran-25-ol-3one), DEPT, CDCl3

Appendix 27: NMR data of compounds **207** and **117**

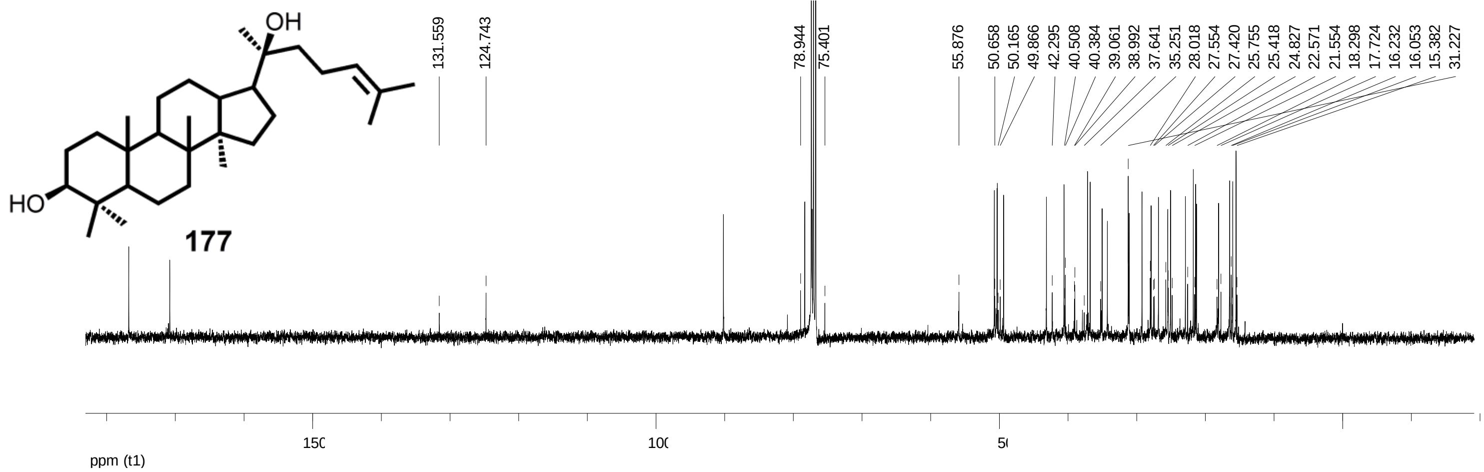
98-66-8C ((3 , 20S)-dammarane lactone acetate), 1H, CDCl3



98-68C ((3 , 20S)-dammarane lactone acetate), 13C, CDCl3

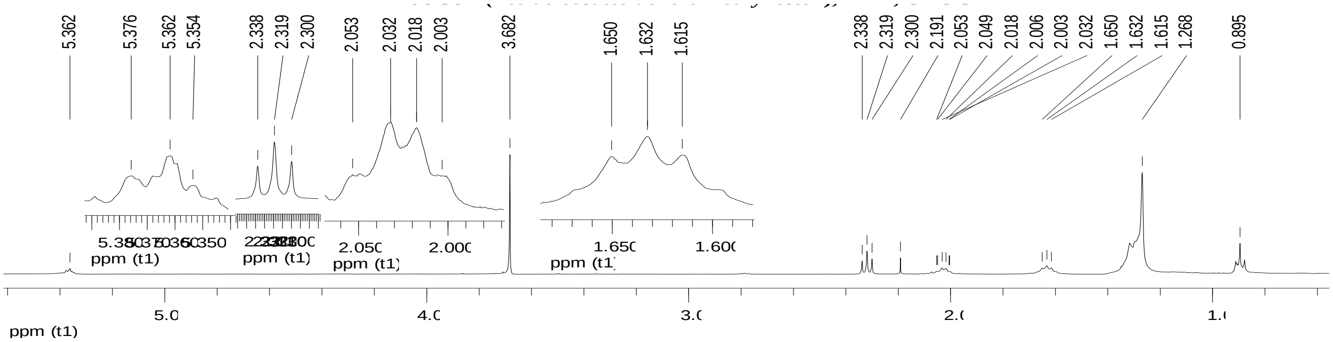


98-68C ((3 , 20S)-dammar-24-endiol, minor), 13C, CDCl3

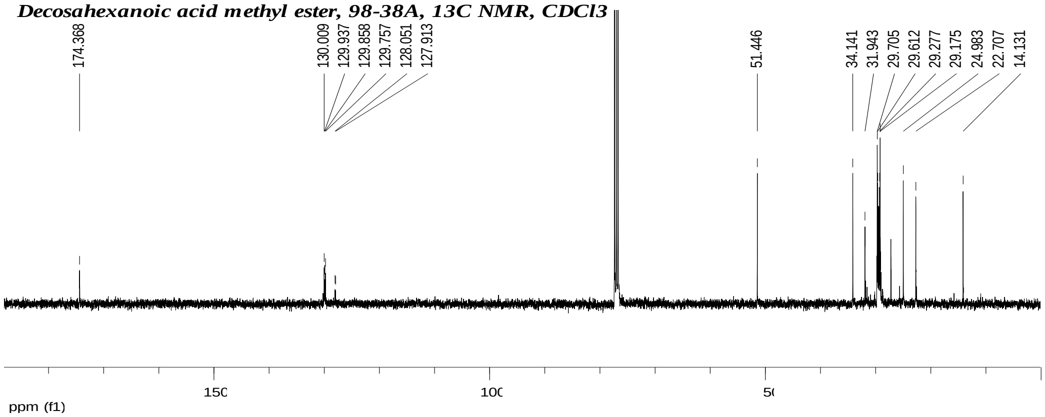


Appendix 28: NMR data of decosahexanoic acid methyl ester (**190**)

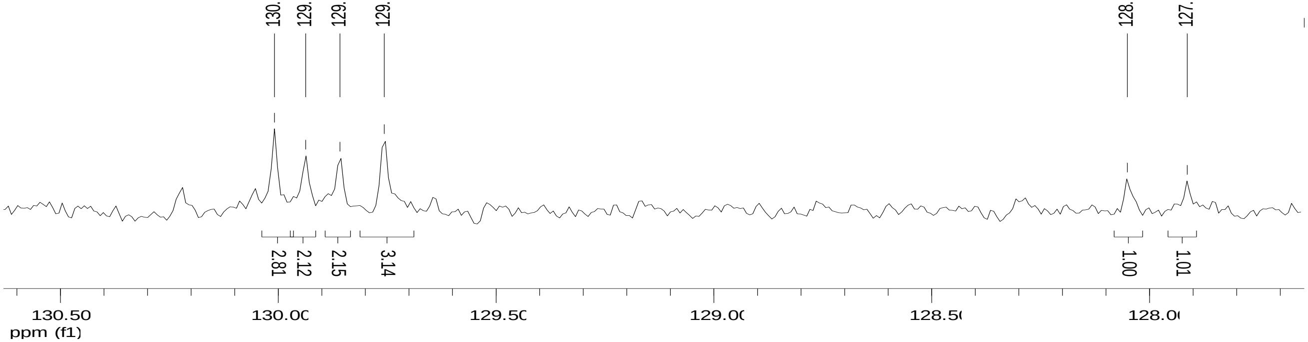
Decosahexanoic acid methyl ester, 98-38A, 1H NMR, CDCl3



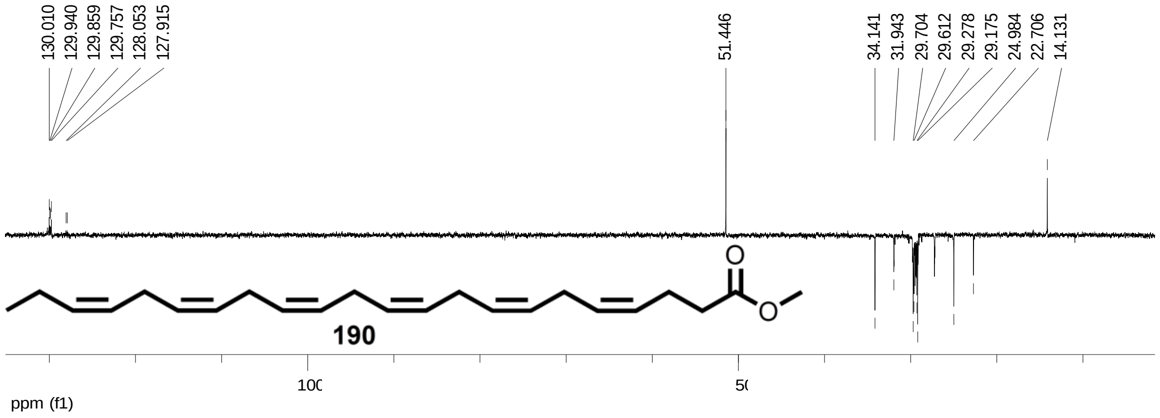
Decosahexanoic acid methyl ester, 98-38A, 13C NMR, CDCl3



98-38A



Decosahexanoic acid methyl ester, 98-38A, DEPT, CDCl3



190