PHYTOCHEMICAL STUDIES ON RESINS OF *BOSWELLIA NEGLECTA* AND *COMMIPHORA SPHAEROCARPA*

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BY

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To my mother Hamu Wari

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ABSTRACT

PHYTOCHEMICAL STUDIES ON RESINS OF *BOSWELLIA NEGLECTA* AND *COMMIPHORA SPHAEROCARPA*

by

Aman Dekebo

Research Advisor: Dr. Ermias Dagne

The family Burseraceae with 17 genera is widely distributed in tropical and subtropical countries. The genus *Commiphora* comprises 150-200 species and *Boswellia* 20 species. Gum exudates of some *Commiphora* and *Boswellia* species have been used for centuries as medicinal and perfumery substances. In the course of this study 7 *Commiphora* and 3 *Boswellia* species were collected and identified. Two of this species are endemic (*B. pirottae* and *C. monoica*). The study on the chemical constituents of two species (*B. neglecta* and *C. sphaerocarpa*) was undertaken.The resin of *C. sphaerocarpa* used as a traditional medicine by the local people is studied chemically for the first time. The extract from the resin of *B. neglecta* yielded as a major component **Bn-8** tentatively identified as 12-uresen-3-ol. The extract obtained from the resin of *C. sphaerocarpa* afforded five compounds coded as **Cs-4**, **Cs-5**, **Cs-6**, **Cs-9** and **Cs-10**. **Cs-6** was identified as the novel compound 6-hydroxy isofuranogermacrene (**84**). Other characterized compounds are **Cs-9** (3-methoxy-10-methylenefuranogermacra-1(2)-en-6-one (**22**) and **Cs-10** (2-methoxyfuranogermacra-1(10)-en-6-one (**20**) previously isolated from the resins of *C. molmol* and *C. holtziana*. Characterization of **Cs-4** and **Cs-5** is in progress.

1. INTRODUCTION

The family Burseraceae with seventeen genera and 500-600 species is widespread in tropical and subtropical countries. Members of the family are often dominant constituents of the vegetation in dry lowland areas. Most of the species yield fragrant olo-gum-resins when the bark is damaged. *Boswellia* Roxb. species yield frankincense, *Bursera* Jacq. and *Canaium* L. Yield elemi, *Commiphora* yields myrrh, bdellium and other resins. In Ethiopia there are only two genera that belong to this family namely, *Boswellia* and *Commiphora.*

The genus *Commiphora* is a very conspicuous and dominant element in dry bushlands. The genus *Commiphora* has 150-200 species. Many species are leafless shrubs or trees for most of the year and usually set flower and fruit when leafless or with young leaves. This makes the collecting of correlated leafy and fertile material difficult [1]. These plants contain many schizogenous ducts in the bark and these may form large interconnecting cavities from which the gum-resin flows freely on wounding or from natural fissures in the bark. The fluid material hardens within 2-4 weeks [2]. The most common commercial product is obtained from *C. myrrh.* Other resin-producing *Commiphora* species occur in southern Arabia, Sudan, Ethiopia, Eritrea, Somalia and Kenya. *C. erythraea* and *C. kataf*, the main sources of opopanax are abundant in southern Arabia, Somalia, eastern Ethiopia and Kenya [3,4].

*Boswellia*has about 20 species in the dry regions of tropical Africa from W. Africa to Arabia and south to NE Tanzania, also in India and one species in Madagascar. The genus is centered in NE Africa where about 75% of the species are endemic to the area [1]. The resin obtained from *Boswellia* species is known in common parlance as frankincense or olibanum. The resins, particularly olibanum, are used in unprocessed form for both fragrance and flavour purposes. The major fragrance uses are burning as incense for home use and also in religious ceremonies. Small amounts of a resin are distilled to yield volatile oils which have their own characteristic balsamic odours used in perfumery. Solvent extracts are also prepared and both resinoids and absolutes are used as fixatives in perfumes. Myrrh was commonly used mixed with frankincense and was a constituent of the celebrated "Kyphi" of the Egyptians used in fumigation and embalming [2]. Gum exudates of some *Commiphora* and *Boswellia* species have been collected for centuries as medicinal substances [3].

Although the history of resins from Burseraceae family dates back since pharaohic times of the ancient Egyptians, it is quite surprising to note that the chemistry of these resins is not well documented. The major components of these resins have been outlined as sesquiterpenoids [5] but as pointed out recently most of the reports in the literature are vague with regards to geographical and botanical source of the resins investigated [6]. Previous investigations of some East African species of the genera *Commiphora* and *Boswellia* have shown that resins of some species contain appreciable amounts of volatile compounds in which monoterpenes or sesquiterpenes predominate [5].

1.1 Myrrh

Myrrh is a natural oleo-gum-resin composed of about 3-8% essential oil, 30-60% water soluble gum, and 25-40% alcohol soluble resin [7]. Myrrh is derived from species of the genus *Commiphora* of the Burseraceae [2]. A number of oleo-gum resins called bdelliums are produced in Arabia and Somalia from various species of Commiphora (*C. africana*, *C. habessinica*, *C. hildebrandti*, *C. kua*, *C. schimperi*, etc) and resemble myrrh and may be used for adulteration today. The true source of myrrh has been considered *C. myrrh* Engl. Consequently there is lack of clarity in the chemistry of myrrh because most of the reports on myrrh are based on analysis of commercial samples which include resins from other species.

Myrrh has been employed for incense and embalming since ancient times [2]. Myrrh is included in formulations of a number of modern perfumes. In flavours, myrrh gives a biting-burning somewhat acrid-aromatic taste for mouth washes and tooth pastes. Myrrh is found in a salve used in treating haemorrhoids and wounds. Internally, myrrh is used for indigestion, ulcers and bronchial congestion [8]. The alcolholic solution after filtration can be concentrated and yields the so called "absolute" which is a useful fixative [7].

The chemical work of Brieskorn and Noble (1980-1983) on myrrh which established the presence of a number of interesting sesquiterpenes is based on material that is vaguely described [9-12]. The work of Mardufu in 1982 is among the first reports on a resin from identified *C. myrrha*. However, much remains to be done on the chemistry of these resins, since in Ethiopia alone there are more than 50 *Commiphora* species [1].

Some attempt has been made to describe the economic botany of myrrh and the allied resins. Much effort is needed to document the ethnobotany of these resins. Ethnobotanical notes by collectors of specimens of *Commiphora* species deposited at the National Herbarium in Addis Ababa University reveal some interesting uses of the resins by different communities in Ethiopia. Thus, the gums *C. kua* and *C. habessinica* are used as soap in Borena. *C. tubuk* and *C. coronillifolia* are used as glue and for making ink respectively. The resins with medicinal values are those of *C. gowlello* used against swelling on people and live stocks, *C. incisa* to treat skin disease, *C. ogadensis* against joint ring worm and *C. myrrha* against joint problems and its smoke to repel snakes and flies.

1.2 Opopanax and Bissabol

Opopanax is another commercial product of *Commiphora*. Commercial gum of opopanax is the sun dried, exudation from the bark of *C. erythraea* [7], a species known to be widely distributed in Ethiopia, Sudan, Kenya, Somalia, north Tanzania and Arabia. *C. holtziana*, now treated as synonym of *C. erythraea* was reported by Provan *et al.* in 1989 to be the source of opopanax in Kenya [13]. Opopanax is known in Ethiopia as *abeked.* Opopanax derived from *C. erythraea* is used as insecticide by the Borena people and it is also used as incenses [4]. Vollesen [1] maintains that *C. erythraea* is known to be a variable species even within small areas; an observation that may have implications on the quality and quantity of opopanax obtained from different localities. The alcoholic solution, after filtration, can be concentrated to yield, the so-called absolute opopanax. Absolute of opopanax is also a very useful fixative [7].

The botanical origin of the other major *Commiphora* resin obtained from Somalia and known as scented myrrh, *Bissabol* (Hindi) and *habak hadi* (Somali) was recently clarified by Thulin and Claeson in 1991 [14]. These workers maintain that this resin is produced by *C. guidotti,* a species with restricted distribution in southern Ethiopia and Somalia. *Bissabol* is exported mainly to China and to European perfume industry as "opopanax," hence the confusion of opopanax with *Bissabol.*

1.3 Frankincense

Frankincense or olibanum is the name given to the natural oleo-gum resins derived from different species of *Boswellia.* The resins comprise 5-10% essential oil, 65-85% alcohol and the remaining water-soluble resins [7].

The major frankincense sources of the world today are Ethiopia, Somalia and north east Kenya. The principal frankincense producing species include *B. papryfera, B. neglecta* and *B. rivae* occurring in Ethiopia, *B. sacra* and *B. frereana* in Somalia and *B. serrata* in India. *B. sacra* which also occurs in Yemen is the Arabian frankincense of classical times and is also known as "Bible incense"[8]. There are other *Boswellia* species that occur sporadically and are used to some extent by local communities. This includes the highly aromatic resin producing *B. pirotae* which is endemic to central and north Ethiopia [1] and *B. microphylla* found in Kenya and

Ethiopia.

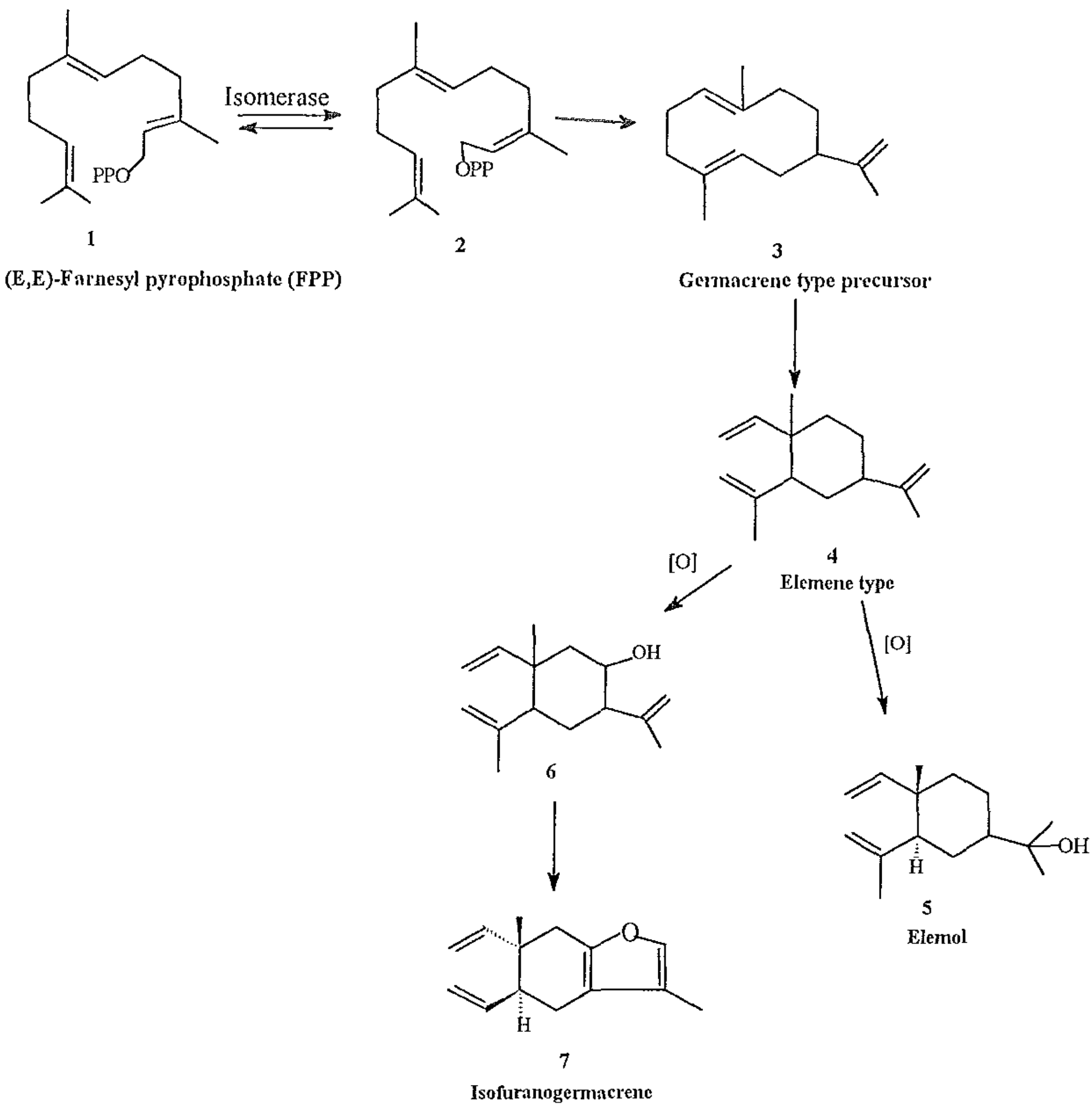
The word frankincense is derived from the French *franc encens,* meaning “pure incense”. Frankincense is known in Arabic as *luban,* in Greek *libanos* and in Ethiopia *itan.* It has a wide use including as incense in homes, formulation of a number of modern perfumes and as medicine [8]. Olibanum is also used as component of adhesive plasters.

1.4 Composition of essential oils of *Commiphora*

A few species of *Commiphora* are noteworthy for containing a highly volatile liquid resin which is expelled as a spray when the branches and woody twigs of the tree are bent and flows out over the surrounding area when the tree is wounded.The fragrance raw material myrrh oil or oil of Heerabol-myrrh is prepared by steam distillation of the resin myrrh. Myrrh absolute is prepared by ethanol extraction of myrrh followed by evaporatioin. The essential oils vary widely in their chemical composition. Most essential oils contain a large number of constituents. The common constituents of the essential oils are monoterpenoid and sesquiterpenoid hydrocarbons and their derivatives [5].

1.5 A possible route of elemol and isofuranogermacrene

The volatile or essential oil constituents of the family Burseraceae are predominantly monoterpenoids and sesquiterpenoids. Elemol and isofuranogermacrene usually co-occur in *C. myrrha.* A possible route to elemol and isofuranogermacrene is given in **Scheme 1.1** [5].



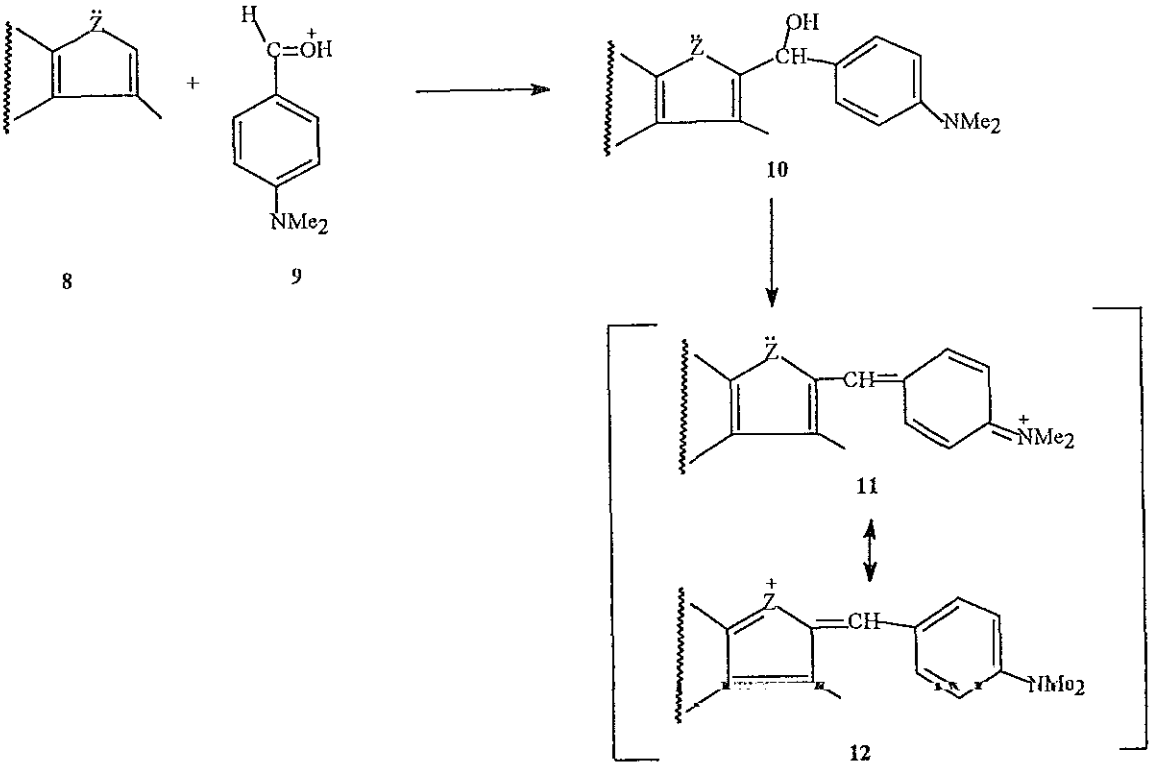
Scheme 1.1 Possible route to elemol and isofuranogermacrene

1.6 An Ehrlich test for furanosesquiterpenes

Reactions of thiophenes, pyrroles and furans with conjugate acids of aldehydes and ketones yield alcohol as the initial product. Usually the alcohol cannot be isolated as such because the hydroxyl group is readily lost to give a cation which is stabilized by resonance and is often the end product. Thus in the Ehrlich test, furanosesquiterpenes react with p-dimethylamino-benzaldehyde and hydrochloric acid to give violet coloured products (**Scheme I.2**) [15-18].

$$p\text{-}Me_2NC_6H_4CHO \xrightarrow{EtOH/HCl} p\text{-}Me_2NC_6H_4CH{=}\overset{+}{O}H$$

p-dimethylaminobenzaladehyde



Z = O, N, S

Scheme 1.2 Reaction of furanosesquiterpenes with Ehrlich reagent

1.7 Characterization

Terpenes and their oxygenated derivatives are the main constituents of the essential oils. These compounds are composed of isoprene units, monoterpenes being composed of two units (C_{10}) and sesquiterpenes of three units (C_{15}) [19,20]. These compounds may have different chemical characteristics and wide boiling point ranges. The best method of separation of individual components is by gas liquid chromatography (GLC).

Oxygenated terpenoids are also the components of essential oils which are responsible for distinctive aroma and flavour. Thus separation and identification of oxygenated terpenoids are of interest to the essential oil and perfume industries [21].

Many terpeniods are unstable. Gas chromatography (GC) separates many of these components and has advantages of being fast, of using an inert atmosphere and of requiring small sample size.

Essential oils are most often separated by steam distillation at atmospheric pressure. The oils are insoluble in water and form an oil phase on the aqueous distillate. The oil layer is drawn off and freed from water. The oils can be separated from a water layer by extraction with petroleum ether. Some essential oil constituents are heat labile and structural rearrangements may take place during isolation by steam distillation at atmospheric pressure. Thus steam distillation at reduced pressure which is less likely to cause changes is some times employed [21].

Under definite set of conditions retention volume or retention time is characteristic of certain components. In many cases the constituents in the mixture can be identified by a simple comparison of their retention volumes with standard substances [22]. The most preferable method used in analysis of essential oils is a gas chromatograph interfaced with a mass spectrometer (GC-MS). Both gas chromatography and mass spectroscopy require sample size in the microgram range. GC-MS provides a facile and sensitive system for identification of complex mixtures [23].

Most compounds can be detected by spray reagents, such as 1% vanillin in sulfuric acid. However, there is no unique test which distinguishes terpenoids as a group from all other components. Some compounds can be viewed under ultraviolet.

Application of spectroscopic techniques such as MS, IR, NMR is usually used together with biogenetic considerations to elucidate structures of terpenoids.

1.8 Objective of the project

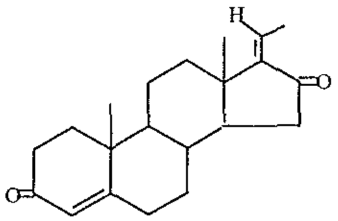
The chemistry of frankincense and myrrh has attracted the attention of several workers for many years. The limitation in the taxonomic and botanical knowledge of the family Burseraceae has hindered chemical work on resins from properly authenticated species. Earlier reports that were based on resins of *Commiphora* and *Boswellia* species obtained from commerce lacked consistency and could not be reproduced. Therefore, much remains to be done on the chemistry of these resins, since in Ethiopia alone there are more than 50 *Commiphora* and six *Boswellia* species [1]. The chemical constituents of most of these species have not yet been investigated. The objective of this project is to make a general survey of *Boswellia* and *Commiphora* species found in Ethiopia, collect specimens that will aid in their botanical identification, gather ethnobotanical information on these species, and collect resins for chemical investigations. In this study due to limitation of time attempts to isolate compounds have been made only on two species *Commiphora sphaerocarpa* Chiov. and *Boswellia neglecta* S. Moore.

2. BRIEF REVIEW OF THE CHEMISTRY AND PHARMACOLOGY OF THE FAMILY BURSERACEAE

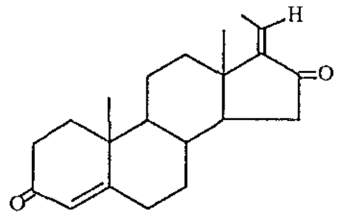
2.1 Chemistry and pharmacology of the genus *Commiphora*

The oleo-gum-resin exudate of the Indian plant, *C. mukul* Engl. is locally known as guggulu gum or Indian bdellium. It is the most widely studied species in the genus. This plant is well known in the Indian traditional medicine system. The system claims guggulu to be efficacious in the treatment of rheumatoid arthritis, obesity and other allied disorders [24].

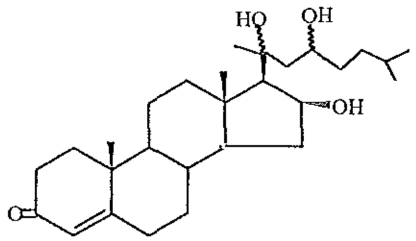
Patil *et al.* in 1972 reported from the resin of *C. mukul* [24] steroidal constituents such as 4,17(20)-(*trans*)-pregnadiene-3,16-dione (**13**), 4,17(20)-(*cis*)-pregnadiene-3,16-dione (**14**), and three new sterols guggulsterol-I (**15**), guggulsterol-II (**16**), guggulsterol-III (**17**) new diterpenoids Cembrene-A (**18**) and mukulol (**19**) [30,31], two long chain aliphatic tetrols octadecan-1,2,3,4-tertrol and eicosan-1,2,3,4- tetrol [32] .



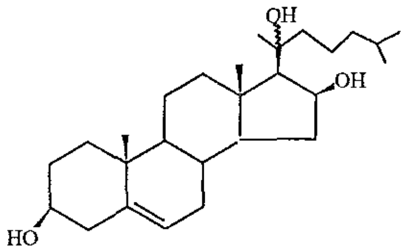
13
4,17(20)-(trans)-pregnadiene-3,16-dione



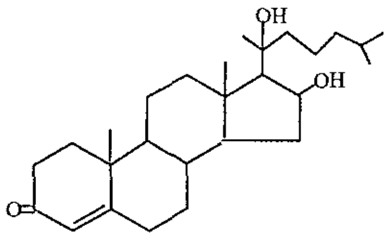
14
4,17(20)-(cis)-pregnadiene-3,16-dione



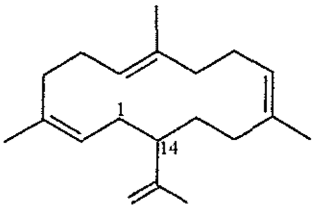
15
Guggulsterol-I



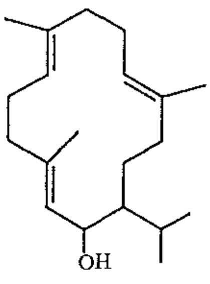
16
Guggulsterol-II



17
Guggulsterol-III



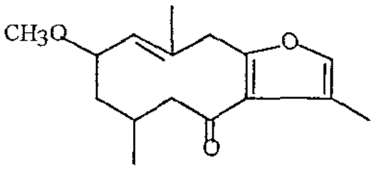
18
Cembrene-A



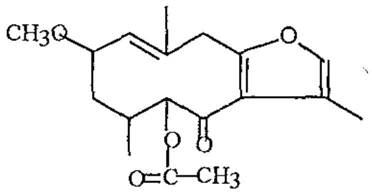
19
Mukulol

Extensive pharmacological studies have been conducted on the crude extract and pure compounds of *C. mukul.* These studies include hypocholesterolemic [25] hypolipdemic [26], anti-inflammatory study effects [27,28,29]. Other pharmacological properties such as anti-atherogenic [33], hypercholesterolemia and hyperlipidemia [34] thyroid gland stimulating [35] and blood lipid lowering effects [36] were also reported for the crude extract and pure compounds of *C. mukul.* Mester *et al.* reported that the steroidal fraction of the gum-resin of *C. mukul* exhibited marked inhibition of ADP, adrenaline and serotonin induced platelet aggregation [37].

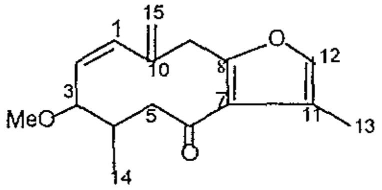
Brieskorn and Noble in 1980-1983 studied the essential oil of myrrh and reported three new furanogermacrens, 2-methoxyfuranogermacra-1(10)-en-6-one (**20**), 5-acetoxy-2-methoxyfuranogermacra-1(10)-en-6-one (**21**), 3-methoxy-10-methylenefuranogermacra-1(2)-en-6-one (**22**) and twelve known sesquiterpenoids, elemol (**5**), furanodiene (**23**), furanodienone (**24**), isofuranogermacrene (**7**), curzerenone (**25**), lindestrene (**26**), furanoeudesma-1,3-diene (**27**) furanoeudesma-1,4-diene-6-one (**28**) 1(10)z, 4z-furanodiene-6-one (**29**), 2-methoxyfurnodiene (**30**), 2-acetoxyfuranodiene (**31**), 4,5-dihydrofuranodiene-6-one (**32**) [9,10,11,12].



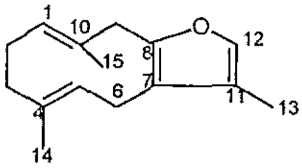
20
2-Methoxyfuranogermacra-1(10)-en-6-one



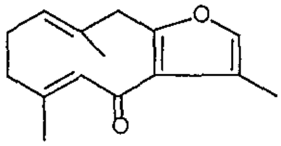
21
5-Acetoxy-2-methoxy-furanogermacra-1(10)-en-6-one



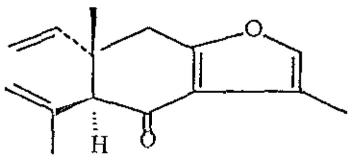
22
3-Methoxy-10-methylenefurano-germacra-1(2)-en-6-one



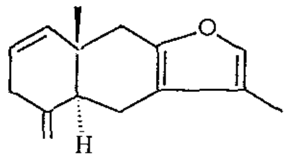
23
Furanodiene



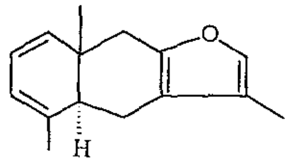
24
Furanodienone



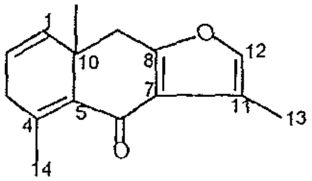
25
Curzerenone



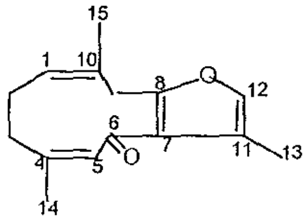
26
Lindestrene



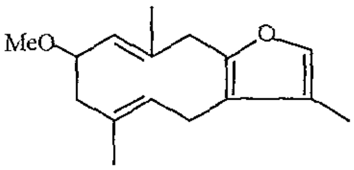
27
Furanoeudesma-1,3-diene



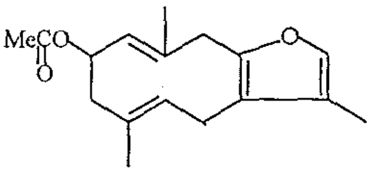
28
Furanoeudesma-1,4-diene-6-one



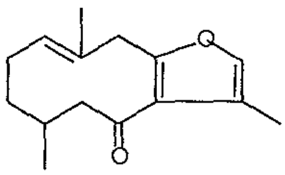
29
1(10)Z, 4Z-furanodiene-6-one



30
2-Methoxyfuranodiene



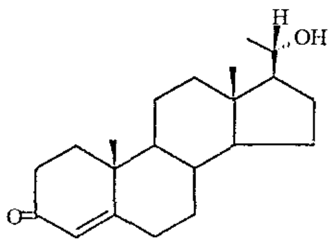
31
2-Acetoxyfuranodiene

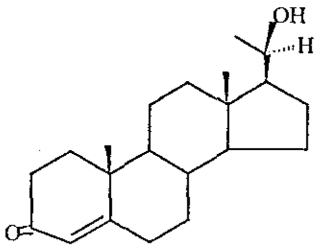


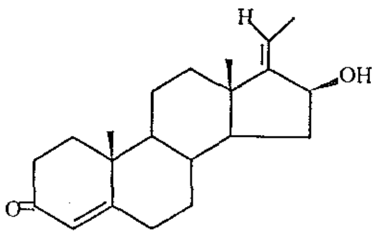
32
4,5-Dihydrofuranodiene-6-one

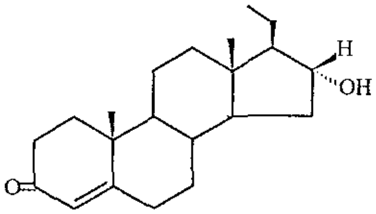
In 1982 Maradufu isolated furanodienone (**24**), 2-methoxyfuranodiene (**30**) and 2-acetoxyfuranodiene (**31**) from an identified resin of *C. myrrha* and also investigated ixodicidal activities of these compounds against *Rhipicephalus appendiculatus* or the tick larvae [6].

In 1982 Bajaj, *et al.* studied the chemical constituents of a resin from *C. mukul* and isolated 20α-hydroxy-4-pregnen-3-one (**33**), 20β-hydroxy-4-pregnen-3-one (**34**), 16β-hydroxy-4,17(20)z-pregnadien-3-one (**35**) and 16α-hydroxy-4-pregnen-3-one (**36**) as new steroidal components of the gum-resin from *C. mukul* [38].


33
20α-hydroxy-4-pregnen-3-one


34
20β-hydroxy-4-pregnen-3-one

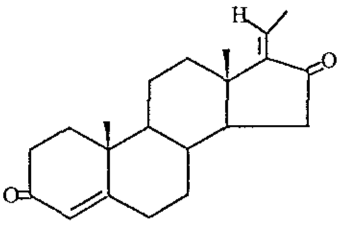

35
16β-hydroxy-4,17(20)Z-pregnadien-3-one


36
16α-hydroxy-4-pregnen-3-one

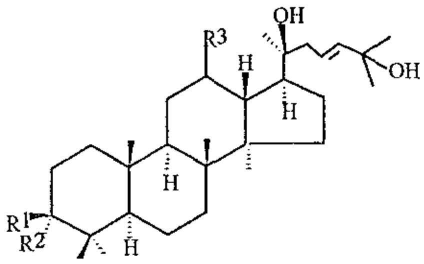
In 1983 Craveiro *et al.* examined the constituents of the gum-resin of *C. guidotti*, (Somali name *habag-hady*), the source of sweet myrrh, growing wild in the southern province of Somalia and isolated seven sesquiterpene hydrocarbons, furanosesquiterpenoid and furanodiene (**23**) [39].

In 1984 Tripathi *et al.* [40] reported a keto steroid Z-guggulsterone (**37**) from the oleogum resin of *C. mukul* which is responsible for the stimulation of a thyroid.

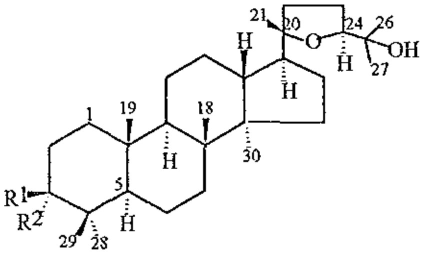
In 1985 phytochemical studies by Waterman and Ampfo [41] on the stem bark of *C. dalzielli* resulted in the isolation of isofouquierone (**38**), cabraleone (**39**), cabraleadiol (**40**) and cabraleadiol-3-acetate (**41**).



37
Z-guggulsterone

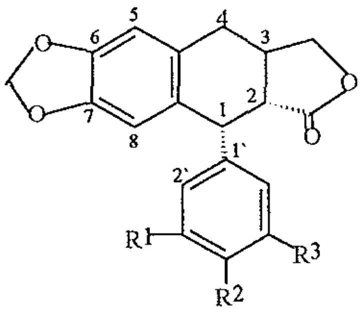


38
$R^1 = R^2 = O$; R^3, $R^4 = H$ Isofouquierone



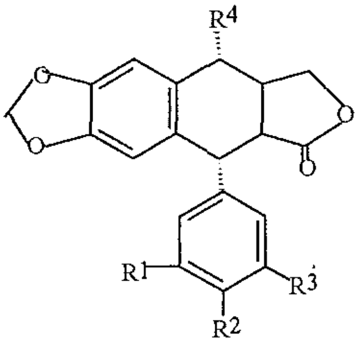
39 $R^1 = R^2 = O$ Cabraleone
40 $R^1 = H$; $R^2 = OH$ Cabraleadiol
41 $R^1 = H$; $R^2 = OAc$ Cabraleadiol 3-acetate

C. incisa Chiov is a heavily spined shrub or tree found in the semi-arid regions of central and northern Kenya. Provan and Waterman in 1985-1988 from a plant first wrongly identified as *C. incisa* which was later found to be *C. kua* reported isolation of epimeric aryltetralin lignans namely polygamain (**42**) and its epimer picropolygamain (**43**), deoxypodophyllotoxin (**44**), picropodophyllin (**45**), mansumbinone (**46**), mansumbinol (**47**), mansumbinone epoxide (**48**), 16(S), 20(R)-dihydroxydammar-24-en-3-one (**49**), mansumbinoic acid (**50**), 1α-acetoxy-9,19-cyclolanost-24-en-3β-ol (**51**), and 29-norlanost-8,24-dien-1α,2α,3β-triol (**52**) [42,43,44].



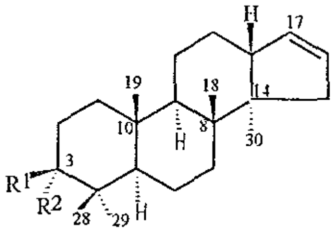
42 $R^1 = R^2 = O\text{-}CH_2\text{-}O$; $R^3 = H$ Polygamain

44 $R^1 = R^2 = R^3 = OCH_3$ Deoxypodophyllotoxin

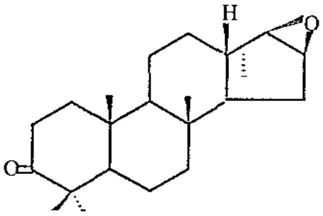


43 $R^1 = R^2 = O\text{-}CH2\text{-}O$; $R^3 = R^4 = H$ Picropolygamain

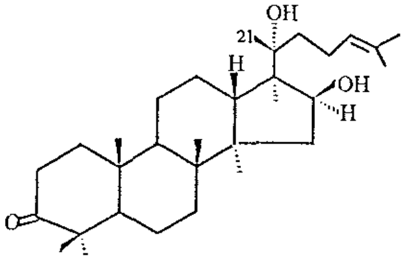
45 $R^1 = R^2 = R^3 = OCH_3$ $R^4 = OH$ Picropodophyllin



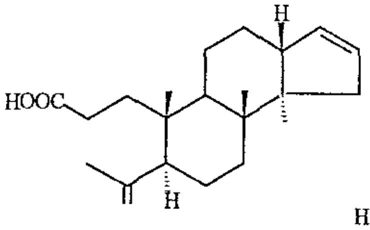
46 R^1, R^2 = O Mansumbinone
47 R^1=OH, R^2 = H Mansumbinol



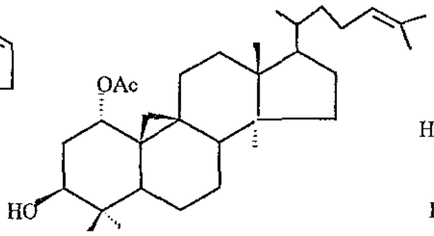
48
Mansumbinone epoxide



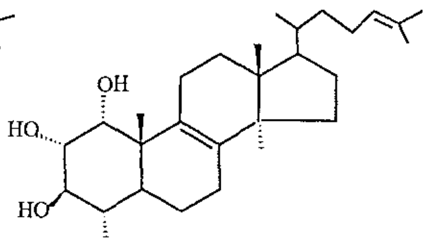
49
16(S), 20(R)-Dihydroxydammar-24-en-3-one



50
Mansumbinoic acid



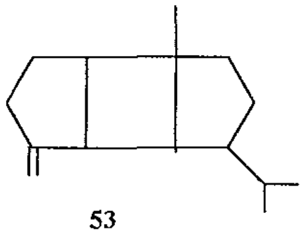
51
1α-acetoxy-9,19-cyclolanost-24-en-3β-ol



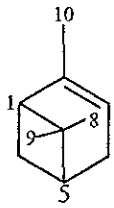
52
29-norlanost-8,24-dien-1α,2α,3β-triol

C. hotziana Engl., to which all Kenyan material previously considered as *C. erythraea* is a large tree characterized by a blue-green bark partially covered with a yellow exfoliating layer. Eighteen sesquiterpenes were isolated from the opopanax oil (*C. erythraea*) [45]. In1987 Provan *et al.* studied the chemical constituents of the resins from *C. myrrha* and *C. holtziana* and isolated furanosesquiterpenes having eudesmane, elamane and germacrane skeletons [13]. These workers reported β-bourbonene (53) from the resin of *C. holtziana.*

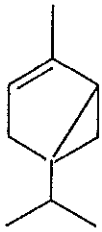
Several species of Burseraceae growing in arid parts of northern and eastern Kenya are characterized by the production of aromatic resins with attractive odours. Provan *et al.* in 1987 [46] examined volatile oils obtained by steam distillation of resins from *B. neglecta*, *C. africana, C. campestris* and *C. ogadensis* and the oils were reported to be entirely monoterpenoid in constitution. All four resins were characterized by having large amounts of α-pinene (54) in their oils where in each case it was generally found to be major component. Other constituents that were important markers of individual species include α-thujene (55) (*B. neglecta, C. africana*), β-pinene (56), myrcene (57) and car-3-ene (58) and limonene (59) (*C. ogadensis*), p-cymene (60), camphene (61), terpinen-4-ol (62), verbenone (63) (*C. africana*).



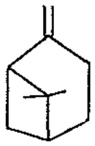
53
β-Bourbonene



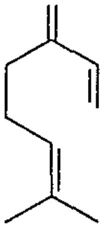
54
α-pinene



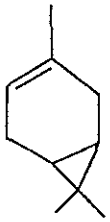
55
α-Thujene



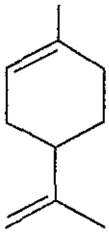
56
β-pinene



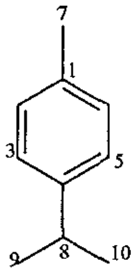
57
Myrcene



58
Car-3-ene

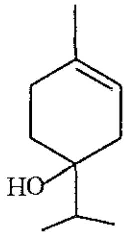


59
Limonene

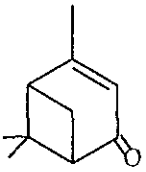


60
P-Cymene

61
Camphene



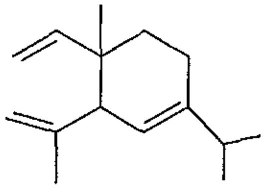
62
Terpinene-4-ol



63
Verbenone

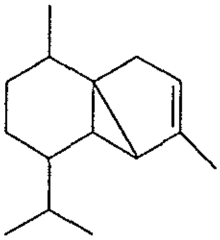
C. rostrata is one of numerous species of the genus found in the arid area of northern Kenya, Somalia and southern Ethiopia. McDowell *et al.* in 1988 [47] examined its volatile resin which exuded from its stem bark and reported oxygenated components. Most *C. rostrata* trees show signs of resin flow but are conspicuous by the absence of herbivore damage or pathogen attack on woody parts. The resin literally sprays from a cut point (caused by bending) which have an overwhelming effect on attacking predators or pathogens. After a short time a white sticky substance forms at the wound site, due to polymerisation of the resin aldehydes and this presumably acts to protect and prevent water loss from the wound. The occurrence of large quantities of aliphatic ketones in exudates has been implicated in chemical defence [47].

A few species of *Commiphora* are noteworthy for containing a highly volatile liquid resin which is expelled when the tree is wounded or when the branches and woody twigs of the tree are bent. *C. terebinthina* Vollesen, widely distributed in northern Kenya and southern Ethiopia, *C. cyclophylla* Chiov., and *C. schimperi* a species of more restricted distribution in southern Ethiopia are some examples of such species. In 1989 Abegaz *et al.* examined the liquid resin obtained on cutting the woody parts of *C. terebinthina* and *C. cyclophylla* and reported monoterpenoid hydrocarbons with limonene (**59**) as major component [48] along with sesquiterpenes such as δ-elemene (**64**) and α-cubebene (**65**) in the oil of *C. terebinthina* and β-elemene (**66**) in both plants oils.



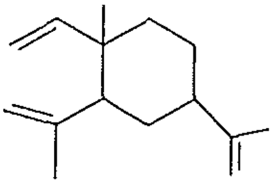
64

δ–Elemene



65

α–Cubebene



66

β–Elemene

Fourie and Snyckers [49] studied the chemical constituents of the root of *C. merkeri* Engl. due to its use in folk medicine against infections. This study revealed a new pentacyclic triterpene with anti-inflammatory and anti-analgesic activity.

Carroll *et al.* [50] reported a repellent and toxicant effects of an extract of *C. erythraea* against ticks [50].

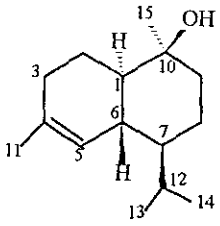
In Somalian traditional medicine the resin of scented myrrh, *C. guidotti* is used against stomach complaints and for the treatment of wounds. Inspired by this traditional medicine Claeson *et al.* isolated a sesquiterpene T-cadinol (67) from the gum-resin of *C. guidotti* and reported its pharmacological activities and confirmed its smooth muscle relaxing and antidiarrhoeal properties [51,52].

Provan *et al.* [53] reported three C_{22} compounds 16-hydroperoxymansumbin-13(17)-en-3-one (**68**), 16-hydroperoxymansumbin-13(17)-en-3β-ol (**69**), 16-hydroperoxy-3,4-seco-mansumbin-3(28),13(17)-dien-3-oic acid (**70**) from the stem bark of *C. kua* which rapidly degraded to give mansumbin-13(17)-en-3,16-dione (**71**), 3β-hydroxymansumbin-13(17)-en-16-one (**72**), and 16-oxo-mansumbin-3(28),13(17)-dien-3-oic acid (**73**).

Duwiejua *et al.* [54] conducted chemical and pharmacological studies on the aqueous extracts of the resins of *B. dalzielli*, *C. incisa*, and *C. mukul.* These studies revealed that the extract of the resins significantly inhibited both the maximal edema response during 6 h of carageeenan-induced rat paw edema. The octanordammarane triterpenes mansumbinone (46) and mansumbinoic acid (50), were isolated from the resin of *C. incisa.* Mansumbinoic acid (50) has been shown to possess considerable anti-inflammatory activity.

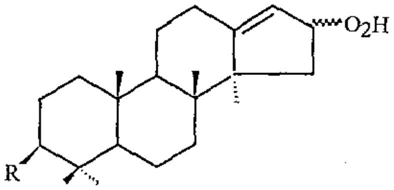
Cavanagh *et al.* in 1993 [55] reported three known and one novel furanogermacrane 1,2-epoxyfurano-10(15)-germacren-6-one (74) from the resin of *C. holtziana.*

There are also other compounds that were previously isolated from the oils, roots and barks of *Commiphora* species as mentioned in Table 2.1.



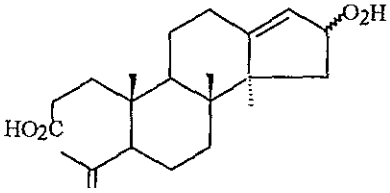
67

T-Cadinol



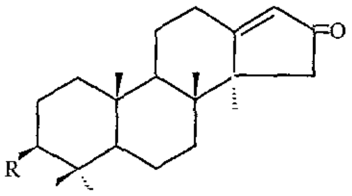
68 R = O 16-Hydroperoxymansumbin-13(17)-en-3-one

69 R = OH 16-Hydroperoxymansumbin-13(17)-en-3β-ol



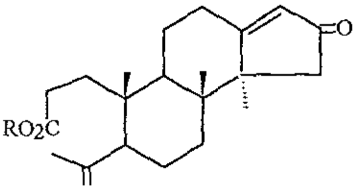
70

16-Hydroperoxy-3,4-seco-mansumbin-3(28),13(17)-dien-3-oic acid



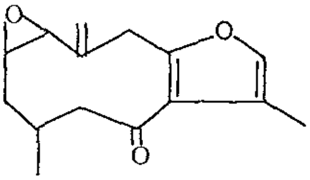
71 R = O Mansumbin-13(17)-en-3,16-dione

72 R = OH 3β-Hydroxymansumbin-13(17)-en-16-one



73

R = H 16-oxo-mansumbin-3(28),13(17)-dien-3-oic acid



74

1,2-epoxyfurano-10(15)-germacren-6-one

Table 2.1: Components of essential oils, resins, root and bark of 15 selected *Commiphora* species.

Species	Compounds	Ref.
C. terebinthina	δ-elemene, longipinene, α-cubebene, β-elemene, t-caryophyllene, β-cubebene, α-muurolene, δ-adinene, α-pinene, sabinene, β-pinene, myrcene, decane, car-3-ene.	48
C. cyclophylla	β-elemene, t-caryophyllene, β-cubebene, α-thujene, α-pinene, sabinene, β-pinene, myrcene, car-3-ene.	48
C. africana	α-thujene, α-pinene, camphene, sabinene, β-pinene, myrcene, car-3-ene, p-cymene, limonene, trpinen-4-ol, verbenone.	46
C. campestris	α-pinene, camphene, sabinene, β-pinene, p-cymene, limonene, terpinen-4-ol, verbenone.	46
C. ogadensis	α-thujene, α-pinene, sabinene, β-pinene, myrcene, car-3-ene, limonene.	46
C. rostrata	2-octanone, 2-nonanone, 2-decanone, 3-undecanone, 2-decanol, 2-dodecanone, 2-undecanol, 2-tridecanone, tridecanal, 2-dodecanol, 2,2-dimethylnonanol, 2-tetradecanone, tetradecanal, hexadecanal, 2,2-dimethylundecanol, 2-pentadecanone, pentadecanal, 2,2-dimethyldecanol, heptadecanal, 2,2-dimethyldodecanol, octadecanal.	47

C. myrrha	β-elemene, γ-elemene, α-cubebene.	13
	2-methoxyfurano-germacra- 1(10)-en-6-one, 5-acetoxy-2-methoxyfurano-germacra-1(10)-en-6-one, 3-methoxy-10-methylenefurano-germacra-1(2)-en-6-one.	9
	elemol, furanodiene, furanodienone, isofuranogermacrene, curzerenone, lindestrene.	10
	2-methoxyfuranodiene, 2-acetoxyfuranodiene, 4,5-dihydrofuranodiene-6-one, 2-methoxyfuranoguaia-9-ene-8-one.	12
	furanoeudesma-1,3-diene, furanoeudesma-1,4-diene-6-one.	11
	commiferin, β-eudesmol.	5
C. holtziana	β-elemene, γ-elemene, elemol, δ-elemene, β-bourbonene.	13
	1,2-epoxyfurano-10(15)-germacren-6-one, 5-acetoxy-2-methoxyfuranogermacra-1(10)-en-6-one, 3-methoxy-10-methylenefuranogermacra-1(2)-en-6-one, 2-methoxyfurano-germacra-1(10)-en-6-one.	55
C. mukul	eicosan-1,2,3,4-tetrol, octadecan-1,2,3,4-tetrol.	32
	cembrene-A, mukulol.	30
	20α-hydroxy-4-pregnen-3-one, 20β-hydrdoxy-4-pregnen-3-one, 16β-hydroxy-4,17(20)Z-pregnadien-3-one, 16α-hydroxy-4-pregnen-3-one.	38
	Z-guggulsterone.	40
	4,17(20)-(cis)-pregnadiene-3,16-dione, guggulsterol-I, guggulsterol-II, guggulsterol-III.	24
	2-hydroxy-4,8,12-trimethyl-1-isopropyl-3,7,11-cyclodecatriene, α-camphene.	31
C. incisa	mansumbinone, mansumbinoic acid.	54

C. guidotti	T-cadinol.	51
	furanodiene, car-3-ene, α-santalene, β-santalene, epi-β-santalene, β-bergamotene, β-farnesene, α-bisabolene, β-bisabolene.	39
C. merkeri	2α,3β-23-trihydroxyolean-12-ene.	49
C. kua	16-hydroperoxymansumbin-13(17)-en-3-one, 3β-hydroxymansumbin-13(17)-en-16-one, 16-oxo-mansumbin-3(28),13(17)-dien-3-oic acid, 16-hydroperoxy-3,4-seco-mansumbin-3(28), 13(17)-dien-3-oic acid, mansumbin-13(17)-en-3,16-dione, 16-hydroperoxymansumbin-13(17)-en-3β-ol.	53
	polygamain, deoxypodophyllotoxin, picropolygamain, picropodophyllin.	42
	1α-acetoxy-9,19-cyclolanost-24-en-3β-ol, 29-norlanost-8,24-dien-1α,2α,3β-triol.	44
	mansumbinol, mansumbinone epoxide, mansumbinone, mansumbinoic acid, 16(S),20(R)-dihydroxydammar-24-en-3-one.	43
C. dalzielii	cabraleone, cabraleadiol, cabraleadiol 3-acetate, isofouqierone.	41
C. erythraea	β-bisabolene, ar-curcumene, β-santalene, humulene, Δ-elemene, α-cubebene, α-copaene, cis-α-bergamotene, β-elemene, α-santalene, trans-α-bergamotene, epi-β-santalene, caryophyllene, γ-elemene, γ-muurolene, trans-α-bisabolene, Δ-cadinene, γ-cadinene.	45

2.2 Distribution and chemistry of *B. neglecta*

Several species of Burseraceae growing in arid parts of northern and eastern Kenya produce aromatic resin with attractive odours. These are produced naturally as small hardened lumps of exudate distributed randomly over the bark or in larger quantities at sites where the bark is damaged. The most important of these species is *B. neglecta* a small tree, branching near the base, with a thick grey-brown bark [1]. *B. neglecta* is also found in Somalia, north east Uganda, north east Tanzania. In Ethiopia it is found in Gamo Gofa, Sidamo, Bale and Hararge [1]. In Kenya the resin of *B. neglecta* is collected and sold commercially (price about 1 US dollar per kg) [46].

In 1987, Provan *et al.*, examined the volatile oil obtained by steam distillation of resin from *B. neglecta*. They reported monoterpenoid constituents of the oil with high concentration of α-pinene (54) [46]. Table 2.2 lists some compouds reported in the oil of *B. neglecta*.

Table 2.2 Some monoterpenes reported in the volatile portion of the resin of *B. neglecta*

Compound	Ref.
α-thujene, α-pinene, camphene, sabinene, β-pinene, car-3-ene, p-cymene, limonene, trpinen-4-ol, verbenone.	46

3. RESULTS AND DISCUSSION

3.1 Specimen collection and identification

In the dry and arid regions of East Africa and South West Africa the Burseraceae in particular the genera *Commiphora* and to a lesser extent *Boswellia* form a major component of the tree flora along with the wide spread *Acacia* (Leguminosae).

Although commercially very important due to the resins they produce not much is known about the botany and chemistry of many members of the genera *Commiphora* and *Boswellia*. The recent botanical report on the Burseraceae of Ethiopia by Vollesen [1] has revealed the occurrence in Ethiopia of 6 *Boswellia* species namely *B. papyrifera*, *B. pirottae*, *B. rivae*, *B. ogadensis*, *B. neglecta* and *B. microphylla*. This worker identified 52 *Commiphora* species in Ethiopia including *C. guidotti* known to produce scented myrrh or bissabol, *C. erythraea* (source of gum opopanax) and *C. myrrha* (the major source of the commercial gum myrrh).

In the course of this project the author made field trips to Metema (Gondar), Blue Nile Gorge (Shoa), Gibe Valley (Kefa), Sof Omar (Bale) Dubluk (Sidamo), Awash National Park (Shoa) and Tis Abay (Gondar). The objectives of the visits were to collect plant specimens for identification and to gather resins for chemical work. At the same time useful ethnobotanical information was recorded by interviewing elders in the communities.

In most cases bark, fruit, leaves and flowers of different species were collected and forwarded to the experienced botanists namely Dr. Sebsebe Demissew (Addis Ababa University) and Dr. Kaj Vollesen (Kew Botanic Gardens, U.K) for identification. Specimens of three *Boswellia* and seven *Commiphora* species were gathered by the author and all were identified by the botanists (Table 3.1).

Table 3.1 List of identified *Boswellia* and *Commiphora* species from which author collected resin for chemical studies

Species	Voucher No.	Place of collection
B. neglecta	D40	Dibluk
B. papyrifera	D5, D15	Abay Valley and Metema
*B. pirottae**	D1, D4, D9, D14	Gibe Valley, Tis Abay and Metema
C. africana	D42	Dibluk
C. cyclophylla	D30	Sof Omar
C. erythraea	D21	Awash National Park
C. habessinica	D39	Dibluk
*C. monoica**	D31	Sof Omar
C. schimperi	D37	Dibluk
C. sphaerocarpa	D32	Sof Omar

* Endemic species of Ethiopia.

3.2 Ethnobotanical information on the collected species

Resins of both genera are widely used as incense, be it at home or in public. Interviews of members of communities in areas where *Boswellia* and *Commiphora* trees grow also revealed that the resins of these species enjoy wide array of traditional uses not only as human medicine but also to treat disease of cattle and camels and as insect repellents. In some instances the fruits, resins and other parts of the plant are used as food additive. Whereas resins of only few species are commercially important, local people however utilize resins from many different species for different purposes. Table 3.2 summarizes the ethnobotanical uses recorded by the author after interviewing several people specifically on resins gathered during the course of this research.

Table 3.2 Brief ethnobotanical information on the collected species

Species	Traditional use
B. neglecta	Resin used for the treatment of skin diseases. It is also used as insect, snake and rat repellent. Bark after boiled used as varnish.
B. papyrifera	Resin used as incense; papery flakes used as rain coat; stem used to prepare furniture.
B. pirottae	Resin used as incense.
C. africana	Root edible.
C. cyclophylla	Resin used as varnish.
C. erythraea	Smoke of its resin used to repel insects.
C. habessinica	Resin used as a soap.
C. monoica	Resin used against skin diseases such as itch.
C. schimperi	Resin for smoothening of skin.
C. sphaerocarpa	Fruit edible; resin used against cough, diarrhoea, headache and against ticks for cattle.

3.3 Investigation of resins of *B. neglecta* and *C. sphaerocarpa*

Although in the course of this research resins of several species were collected, due to limitation of time, we planned to investigate chemically resins of only two species namely *B. neglecta* and *C. sphaerocarpa*. The results of these studies are presented below.

3.3.1 Physical characteristics of resin of *B. neglecta*

This species is known to occur in the southern provinces of Bale, Gamo Gofa, Hararghe and Sidamo. It is also known to occur in Kenya, Somalia, Tanzania and Uganda. In Sidamo it is called "Dakara". It produces a commercially important olibanum which is coloured either black or white. The gum exudes out of the trunk of the tree during the dry season in most cases without incision. However incising the bark by knife is also a common practice. The white resin comes out of the black gum during seasons when the temperature is high. The black gum is produced by each tree more than the white. It is believed that the white gum which costs more is more aromatic than the black but this has not been substantiated by any study. In Dibluk (Sidamo) the black gum may be purchased at *ca* 2 Birr/kg while the white costs *ca* 5 Birr/kg.

3.3.2 Physical appearance and traditional uses of resin of *C. sphaerocarpa*

C. sphaerocarpa is found in Bale, Hararghe, Sidamo and Somalia [1]. Local name: tree "Hagarsu", resin "Habkurto" and fruit "Kechama" (Oromo). When the bark is incised, whitish highly aromatic fluid flows out and crystallizes after three days to yellowish gum. The resin also oozes out from the bark naturally.

The resin is widely used by the local people as a traditional medicine. It is taken orally for the treatment of diarrhoea and headache and its smoke is used to treat cough. The resin is applied with water to treat skin diseases of humans and to rid cattle of ticks.

3.3.3 Isolation and analysis of the essential oil of *B. neglecta*

Hydrodistillation: The ground black resin of *B. neglecta* (300 g) was hydrodistilled. The distillate was dried with anhydrous sodium sulphate and concd under red. pres. to give (13.6 g, 5%) of a yellowish aromatic oil.

Physical data: The following physical data were obtained for the oil.

Optical rotation $[\alpha]_D^{20}$ neat = 25^0

Sp. gravity (d^{20}) = 0.84

Refractive index (n_D^{20}) = 1.46

GC-Analysis of the essential oil:

The oil of black resin of *B. neglecta* was analysed by GC. The GC profile shown in Fig.3.1 was co-injected with some standard monoterpenes. Physical data were also measured for the oil. This result indicated the monoterpenoid constituents of the oil. The major component of the oil was found to be α-pinene (**54**) by GC analysis (co-injection).

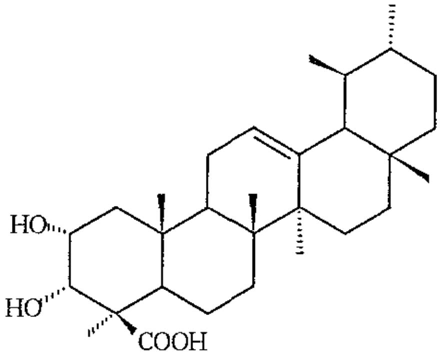
3.3.4 Analysis of the resinoid of *B. neglecta*

The black resin (100 g) was extracted twice with petrol (200 ml) using sonic bath (15 min) to yield 36 g (36 %) of yellowish resinoid. Further extraction with EtOAc and MeOH yielded 20 g (20 %) and 7 g (7%) of brownish extracts respectively bringing the total organic solvent soluble portion to over 60%.

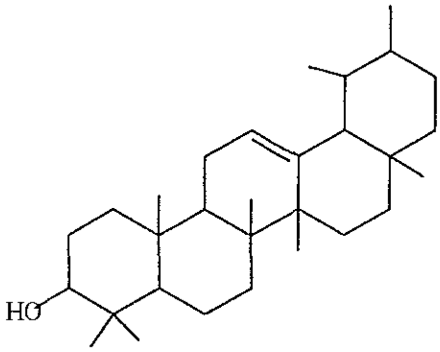
The above resinoid (15 g) was applied on silica gel and eluted with petrol-EtOAc gradients. The fraction obtained from 95:5 (petrol-EtOAc) after PTLC (solvent system II) gave 78 mg of nearly pure compounds quite soluble in petrol and whose structure was partially elucidated as discussed below.

Partial characterization of the major component (Bn-8) of the resinoid of *B. neglecta*

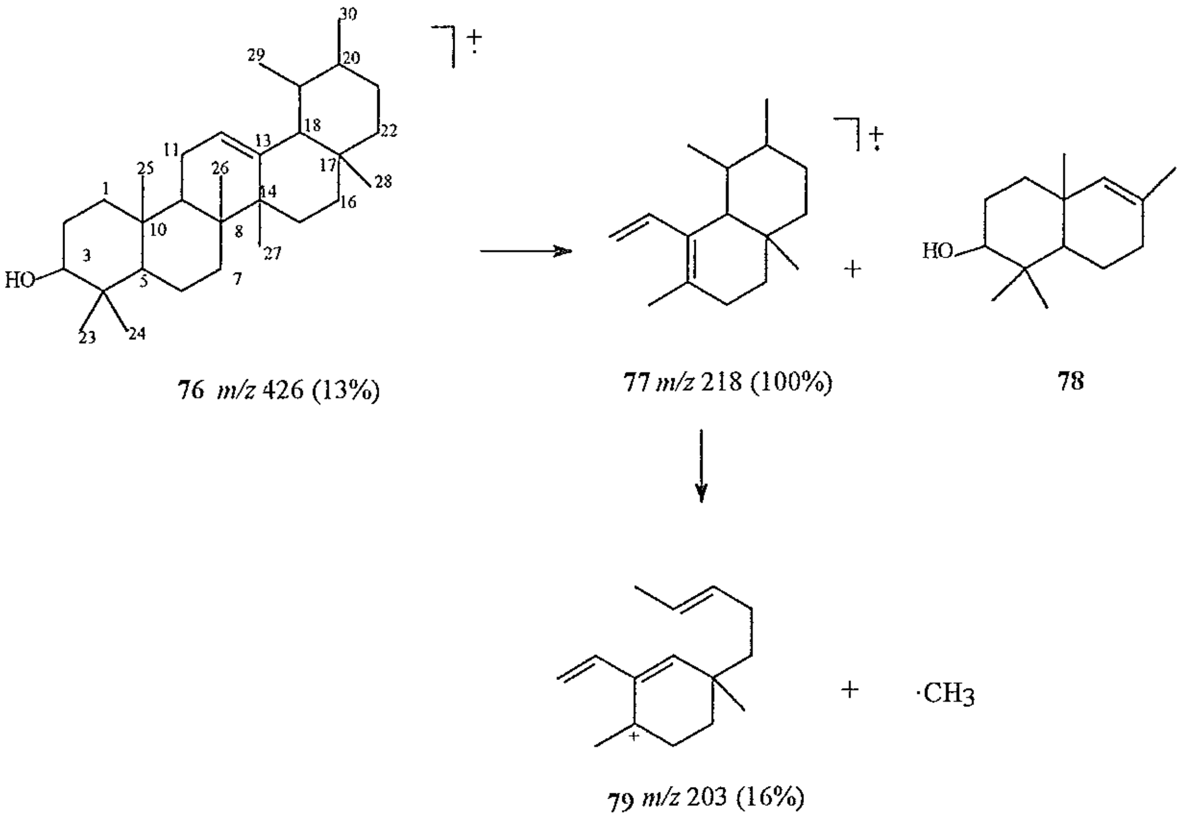
The ^{1}H NMR (300 MHz, $CDCl_3$) spectrum of **Bn-8** is typical of an ursane skeleton. In its ^{1}H NMR spectrum **Bn-8** displayed a multiplet at δ 3.72-3.74 for the methine proton H-3. The signal at δ 5.35-5.42 was assigned to the olefinic proton H-12. The methyl signals of an ursane skeleton were observed at their normal position δ 0.80-1.15. The ^{13}C NMR (75 MHz, $CDCl_3$) spectrum along with DEPT experiment indicated 30 carbon resonances, corresponding to eight CH_3 , nine CH_2, eight CH and the remaining are quaternary carbons. The ^{13}C NMR of the compound was tentatively assigned by comparing it with the ^{13}C NMR data of 2α,3α-dihydroxy-urs-12-ene-24-oic acid (75) isolated from the resin of *B. serrata* [57]. In ^{13}C NMR of the isolated compound, down field shifted signal at δ 124.9 was assigned to C-12 (for complete tentative assignment of the ^{13}C NMR see Table 3.3).The above spectral information support 12-ursen-3-ol (76) as a most probable structure for the compound. The mass spectrum of the compound isolated from the resinoid showed the [M^+] at *m/z* 426 and a base peak at *m/z* 218, along with a strong peak at *m/z* 203 due to a classical retro-Diels fission, diagnostic of olenanes or ursanes [57, 58]. The fragmentation pattern of **Bn-8** is shown in **Scheme 3.1**. This compound was previously isolated from *Artemisia vulgaris* and *Lactuca denticulata* species and is known as α-amyrin (12-ursen-3β-ol) [59].



75
2α,3α-dihydroxy-urs-12-ene-24-oic acid



76
12-ursen-3-ol



Scheme 3.1 EIMS fragmentation pattern of **Bn-8**

Table 3.3 ^{13}C NMR (75 MHz, $CDCl_3$) spectrum of 12-ursen-3-ol (76) and 2α,3α-dihydroxy-urs-12-ene-24-oic acid (76)

Carbon	Chemical shifts (δ_c) 76	 75
1	33.7	41.2
2	41.9	69.0
3	76.6	70.5
4	47.4	47.5
5	47.9	54.9
6	31.7	
7	28.5	31.0
8	41.5	42.4
9	47.5	49.0
10	39.6	38.8
11	18.7	24.2
12	124.9	129.5
13	139.5	142.5
14	41.8	43.4
15	25.7	28.0
16	23.9	26.5
17	33.7	34.0
18	59.5	58.5
19	40.0[a]	37.0
20	40.1[b]	
21	26.9	29.0
22	33.2	39.5
23	15.9	14.0
24	29.2	181.6
25	22.8	21.5
26	17.9	14.5
27	23.9	23.0
28	21.8	18.0
29	28.7	23.5
30	18.7	17.0

a and b may be interchangale.

3.3.5 Chemical studies on resin of *C. sphaerocarpa*

Steam distillation: The resin of *C. sphaerocarpa* (100 g) was hydrodistilled to give slightly yellowish coloured oil (2 g, 2%). The GC analysis of the oil resulted in the chromatogram shown in Fig. 3.2.

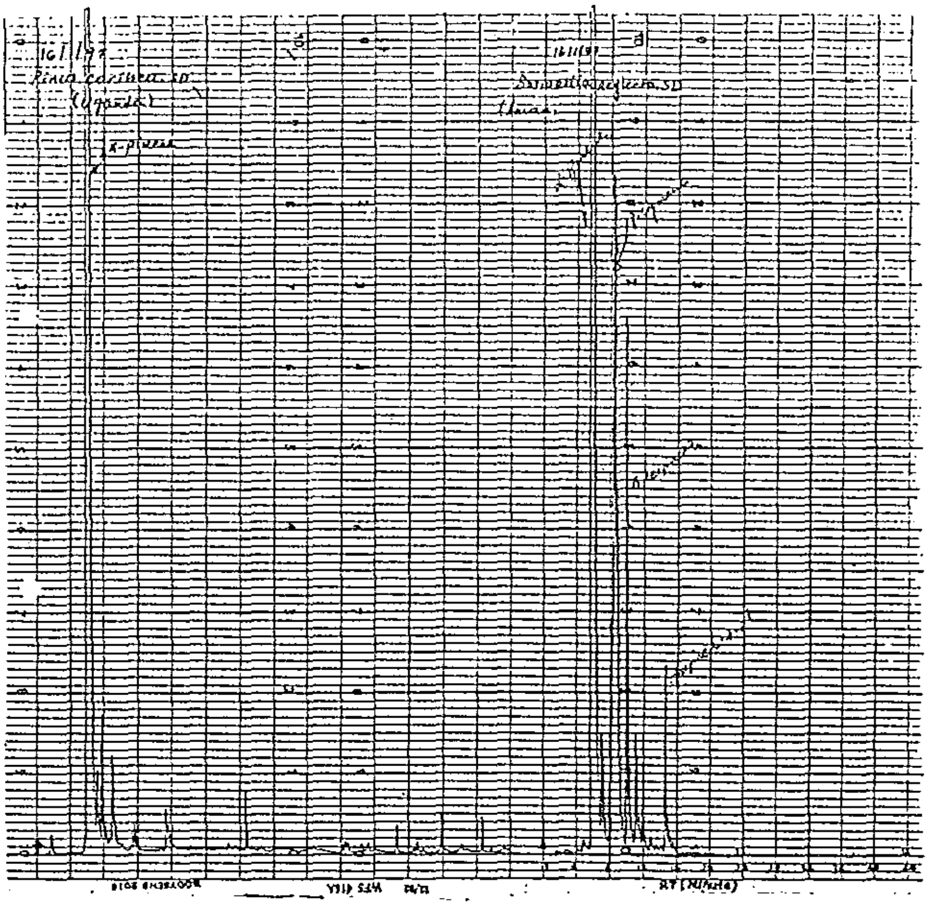


Fig.3.1 GC Chromatogram of (a) α-pinene and (b) essential oil of *B. neglecta*

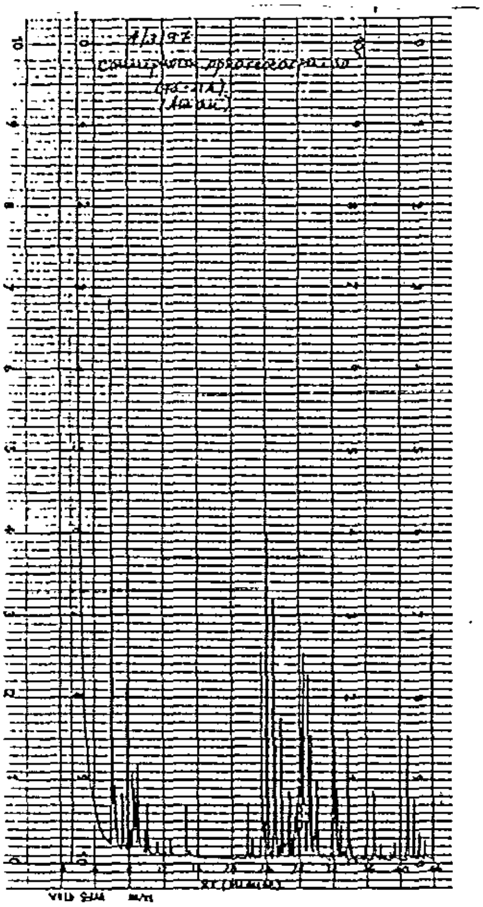


Fig. 3.2 GC Chromatogram of essential oil of *C. sphaerocarpa*

As shown in the GC the components with shorter retention times 6-16 rt. (min) are most likely monoterpenes whereas those with longer retention time are sesquiterpenes. The major peak is indicated to be α-pinene (54) (GC co-injection). However this needs to be confirmed by isolation and generation of spectroscopic data.

Physical data:

The following data were collected for the oil.

Optical rotation neat $[\alpha]_D^{20} = -262^0$

Sp. gravity $(d^{20}) = 0.94$

Refractive index $(n_D^{20}) = 1.5085$

Extraction: The resin (20 g) was extracted with petrol at room temp. to give yellowish gum (2 g, 11%). The extract gave positive test with Ehrlich reagent indicating presence of furanosesquiterpenes. The above petrol extract (2 g) was subjected to CC over silica gel (70 g) and eluted with solvent system V. A total of 33 fractions each 100 ml were collected. The combined fractions of 4 and 5 yielded **Cs-4** (10 mg). The combined fraction 17-19 (40 mg) which was further purified by PTLC using solvent system II displayed 3 bands. **Cs-5** (10 mg) which was obtained from the first band. Fraction 20 gave **Cs-6** (90 mg). Fractions 26-29 were combined to afford **Cs-9** (80 mg). When 2 g EtOAc fraction of the resin was applied on CC eluting with petrol-EtOAc mixtures several fractions were collected. The combined fractions of 24 and 25 were further purified by PTLC (solvent system III) to afford **Cs-10** (15 mg).

Thus nearly pure substances coded **Cs-4**, **Cs-5**, **Cs-6**, **Cs-9** and **Cs-10** were obtained in reasonable amounts. Fig. 3.3 shows the TLC profile of components found in the above fractions.

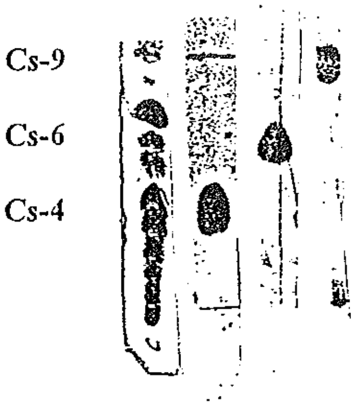
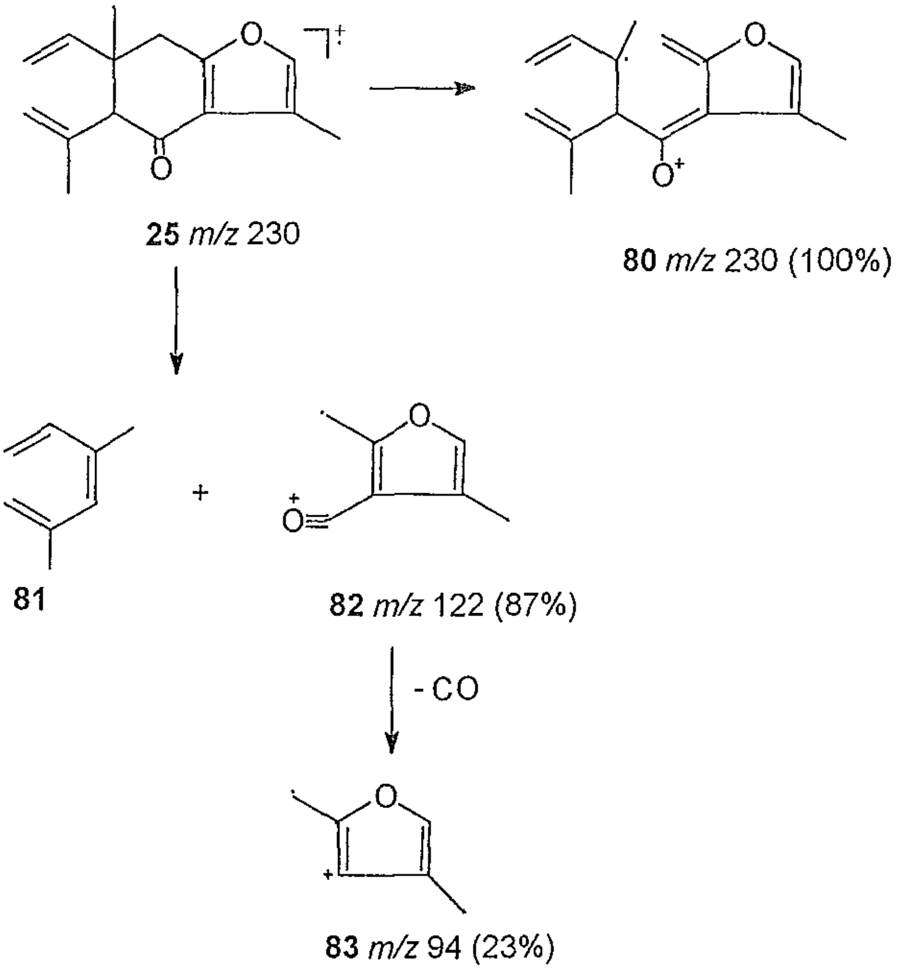


Fig. 3.3 TLC chromatogram of petrol extract and fractions of resin of *C. sphaerocarpa*

In this thesis characterization of 3 components coded **Cs-6**, **Cs-9** and **Cs-10** will be reported. Work is in progress with the characterization of the other components.

Characterization of Cs-6 as the novel compound 6-hydroxyisofuranogermacrene (84): The ^1H and ^{13}C NMR spectra of **Cs-6** shown in Fig. 3.4 indicated that this compound possesses 15 carbons comprising 5 quaternary, 4 methine, 3 methylene and 3 methyl groups (DEPT). The ^1H NMR indicated presence of furano moiety with an α-proton at δ 7.06 (br s) substituted with a methyl group δ 0.8 at the β position consistent with the positive Ehrlich reaction (Fig. 3.4). The two olefinic methylene protons at δ 112.9 and 119.2 (DEPT) suggested an isofuranogermacrene where there is no bonding between C-2 and C-3 (Table 3.4).

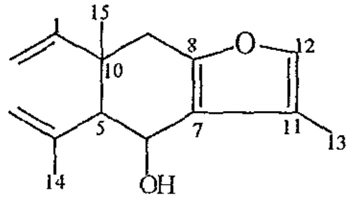
The MS gave a parent ion at *m/z* 230 indicative of a keto group at either C-6 or C-9 (**Scheme 3.2**). However as there was no signal for C=O group in the ^{13}C spectrum, the MS must have resulted from oxidation of the sample on standing. This was shown to be the case by comparing the ^{13}C NMR spectrum of the **Cs-6** sample for which MS was measured with fresh sample.



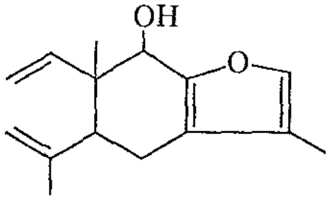
Scheme 3.2 EIMS fragmentation pattern of curzerenone (**25**)

The former exhibited a carbonyl signal at δ 194.9 and the later could also be oxidized readily with pyridine chlorochromate (PCC) to give rise to a keto group (positive DNP test). The above result suggest **CS-6** to be compound in the novel 6-hydroxyisofuranogermacrene (**84**) and its oxidation product to be curzerenone (**25**).

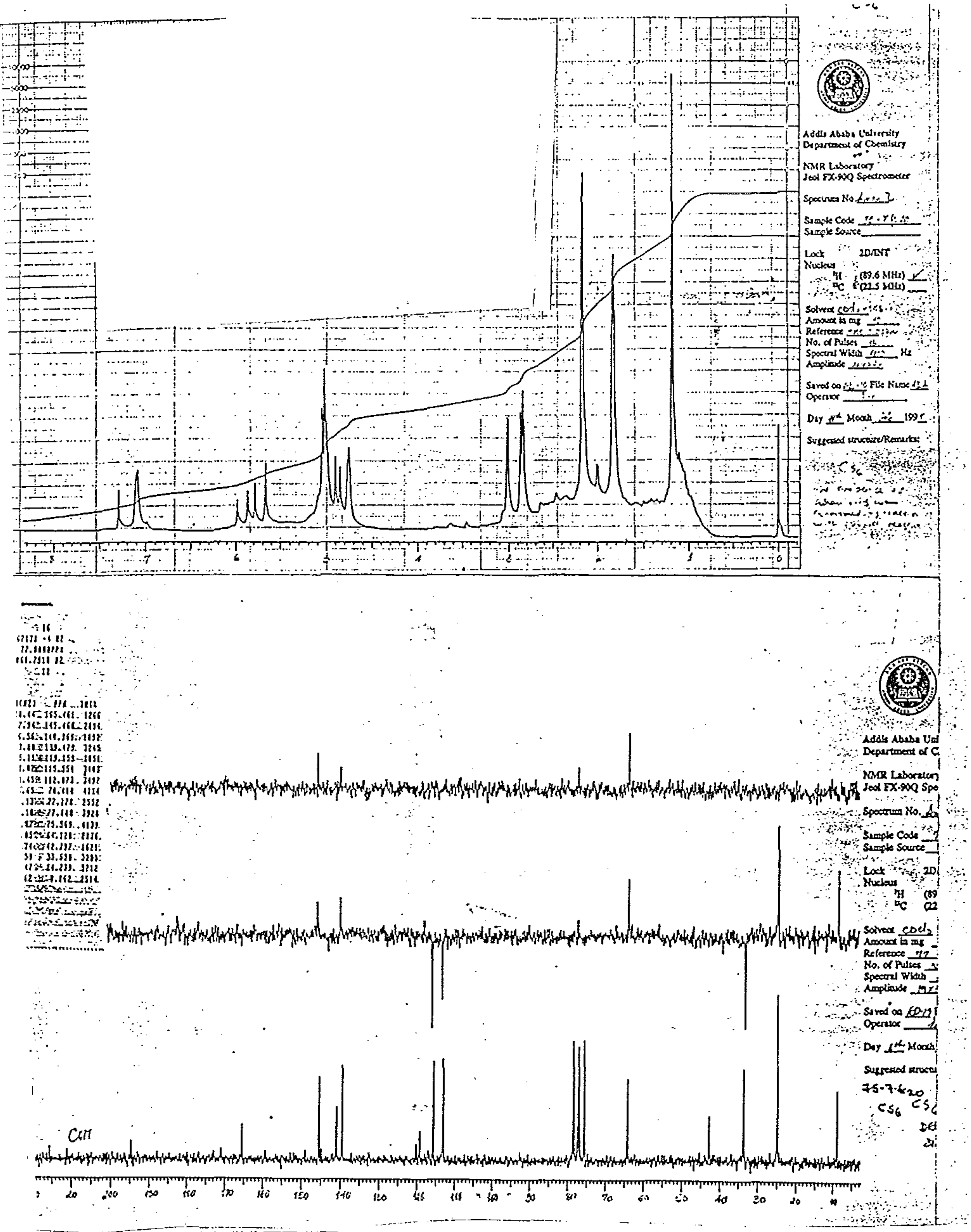
Confirmation by NMR experiments to rule out the alternative 9-hydroxy structure (**85**) is still pending.



84
6-Hydroxyisofuranogermacrene



85
9-Hydroxyisofuranogermacrene



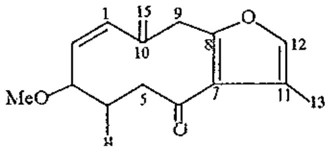
Addis Ababa University
Department of Chemistry
NMR Laboratory
Jeol FX-90Q Spectrometer
Spectrum No.
Sample Code
Sample Source
Lock 2D/INT
Nucleus
1H (89.6 MHz) ✓
13C (22.5 MHz)
Solvent $CDCl_3$
Amount in mg
Reference
No. of Pulses
Spectral Width Hz
Amplitude
Saved on File Name
Operator
Day Month 199
Suggested structure/Remarks:
Cs6

Fig. 3.4 ^{1}H and ^{13}C NMR of Cs-6

Characterization of Cs-9 as 3-methoxy-10-methylenefuranogermacrene (22)

The ^{1}H NMR spectrum of **Cs-9** showed quartet at δ 6.99 which indicated the presence of α-proton of the furan ring. The ^{13}C NMR spectrum indicated the presence of 16 carbon atoms. DEPT spectrum showed that there are 5 quaternary carbons, 5 methine, three methylene and 3 methyl groups. One of the methylene group was exomethylene and one of the quaternary carbons is a keto group. The ^{13}C NMR spectrum demonstrated that **Cs-9** has the furanogermacrene skeleton (Table 3.4). The ^{1}H NMR confirmed the presence of exomethylene (δ 4.8-5.4), OMe (δ 3.25), and the 2 signals for methyl at δ 1.98 and 1.15 indicating these to be C-13 (furanomethyl) and C-14 respectively (Fig. 3.5).

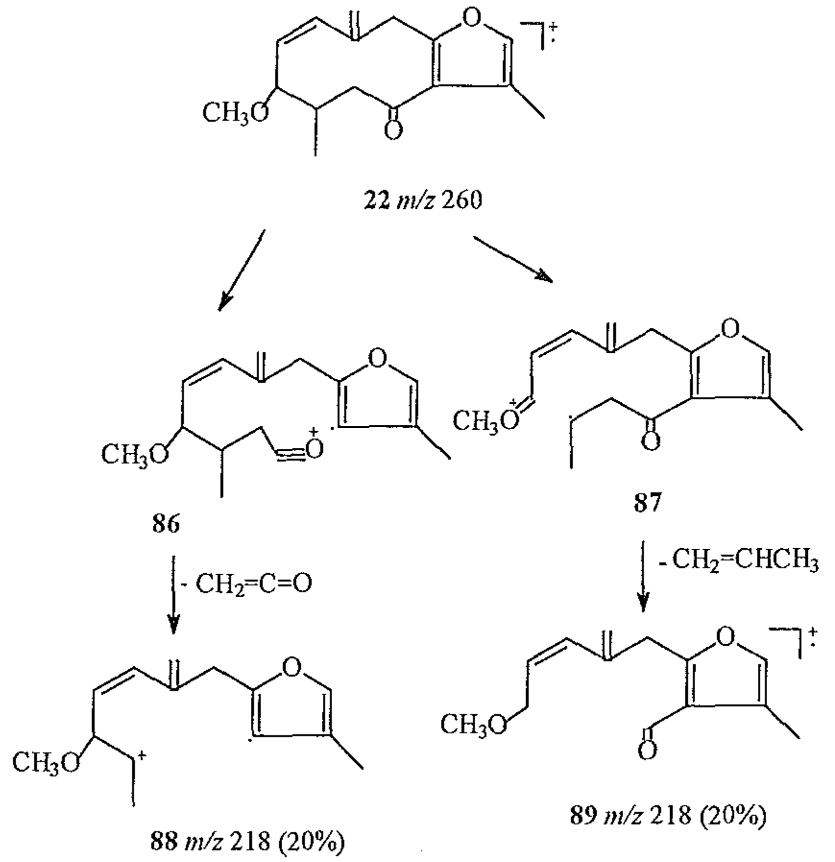
Reaction of the compound with Ehrlich reagent gave a red violet solution (positive test for the presence of the furano group). The presence of the carbonyl group in the compound was confirmed by the positive test of the reaction of the compound with 2,4-dinitrophenylhydrazine reagent. The above results strongly suggest the structure of the compound to be **22**.



22

3-Methoxy-10-methylenefuranogermacra-1(2)-en-6-one

In the MS, fragmentation of the compound to loss neutral compounds supports the proposed structure (**Scheme 3.3**).



Scheme 3.3 Mass fragmentation pattern of 3-methoxy-10-methylenefuranogermacra-1(2)-en-6-one (**22**)

Compound **22** was first isolated by Brieskorn and Noble [9] from the essential oil of myrrh. This compound was also isolated from *C. holtziana* along with a novel sesquiterpene, 1,2-epoxyfurano-10 (15)-germacren-6-one (**74**). This workers corrected ambiguous ^{13}C NMR assignments of the compound in the previous study. Ambiguities relating to C-1 and C-2, C-5 and C-9 were resolved using Heteronuclear Multiple Bond Coherence (HMBC) techniques.

In our study, the NMR spectra of the compound was in good agreement with those reported in the literature.

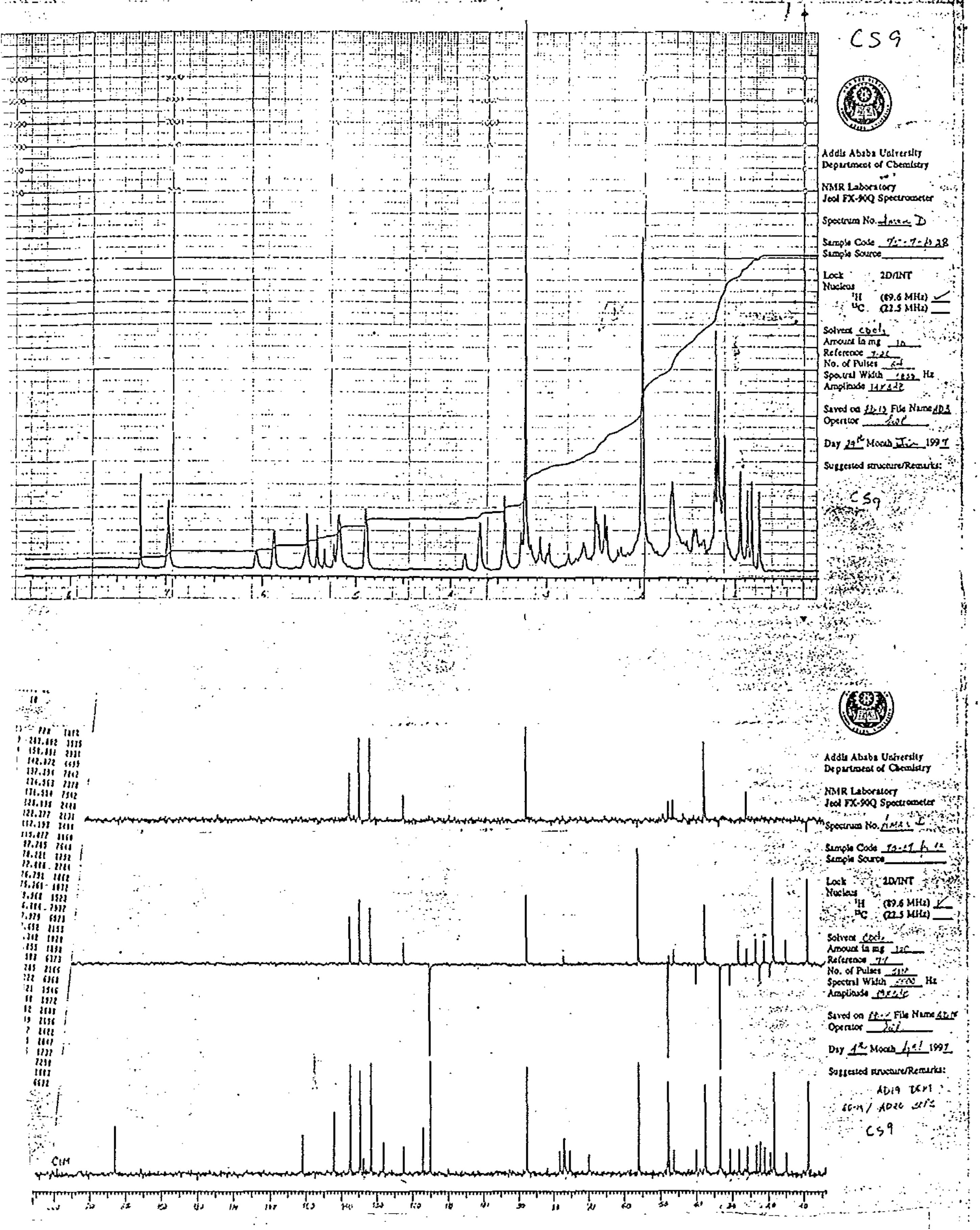
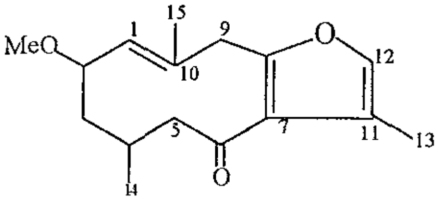


Fig. 3.5 ¹H and ¹³C NMR spectra of Cs-9

Characterization of CS-10 as 2-methoxyfuranogermacra-1(10)-en-6-one (20)

The formula $C_{16}H_{22}O_3$ could be deduced for **Cs-10** from ^1H, ^{13}C including DEPT spectral data. These spectra reveal presence of OMe and C=O functional group, a furano moiety, an exomethylene group and two olefinic protons, suggesting the compound to have structure **20**. Compound **20** was first isolated from the essential oil of *C. molmol* [9].



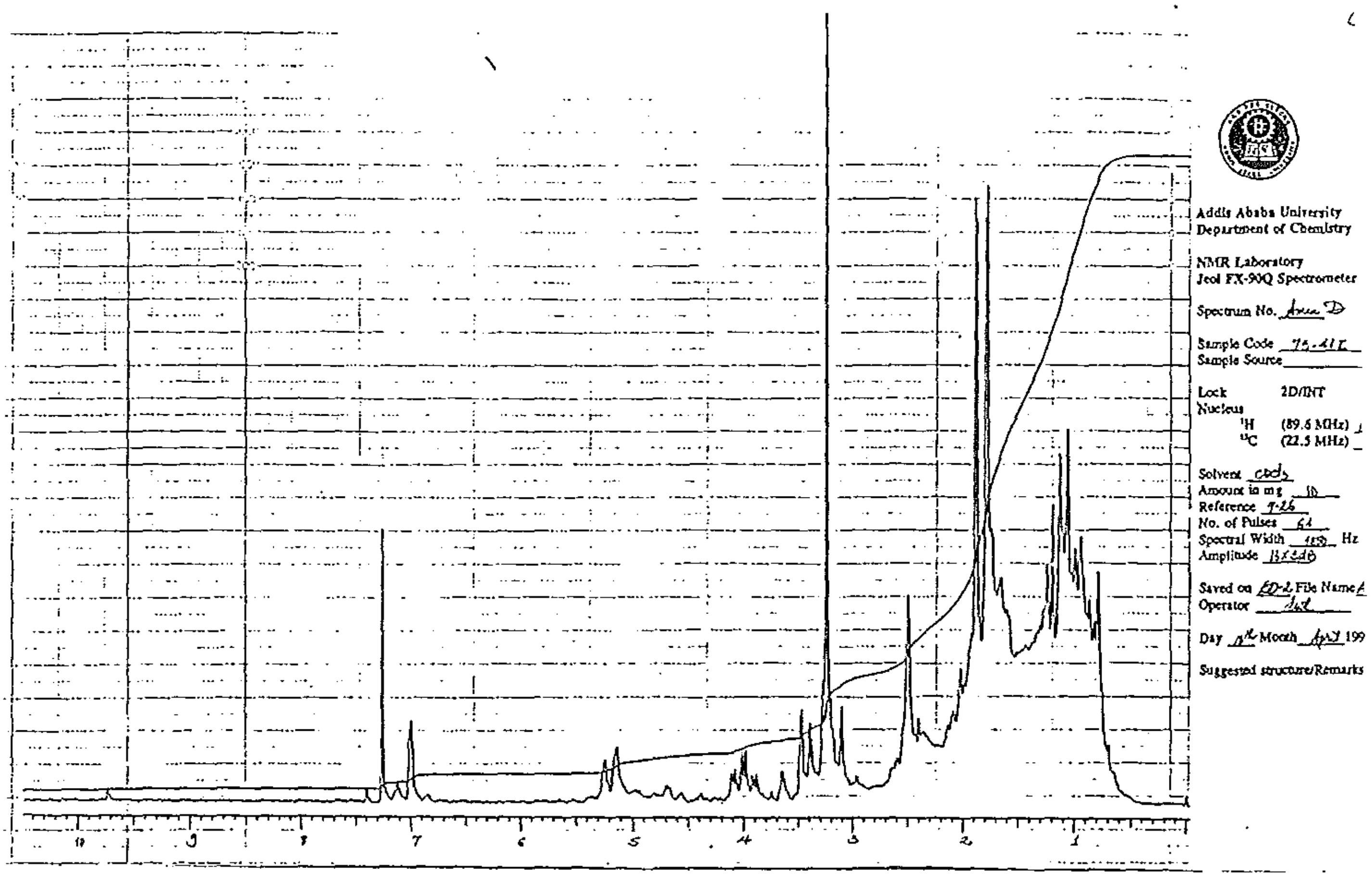
20

2-Methoxyfuranogermacra-1(10)-en-6-one

In the ^1H NMR (br s) at δ 7.0 was assigned for the α-proton of the furan ring. The proton signal appeared at δ 5.20 indicated the olefinic proton. The proton signal (dt) at δ 4.0 was assigned to C-2 proton coupled with both H-2 and H-3. Strong singlet at δ 3.25 was a characteristic signal for CH_3O group protons. The three methyl group protons appeared at δ 1.88, 1.77 and 1.09 were assigned for β-methyl group, C-15 methyl protons and C-14 methyl protons respectively (Fig. 3.6). The ^{13}C NMR support the structure of the compound (Table 3.4).

This compound was also isolated from the resin of *C. holtziana* [55]. Correction to the original reported ^{13}C NMR assignment was made for C-14 and C-15.

The NMR data of this compound was compatible with the previous report. Therefore, **Cs-10** was identified as 2-methoxyfuranogermacra-1(10)-en-6-one (20)


Addis Ababa University
Department of Chemistry

NMR Laboratory
Jeol FX-90Q Spectrometer

Spectrum No. Ann D

Sample Code 75-41E
Sample Source

Lock 2D/INT
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^1H (89.6 MHz)
^{13}C (22.5 MHz)

Solvent $CDCl_3$
Amount in mg 10
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Amplitude 13×2dB

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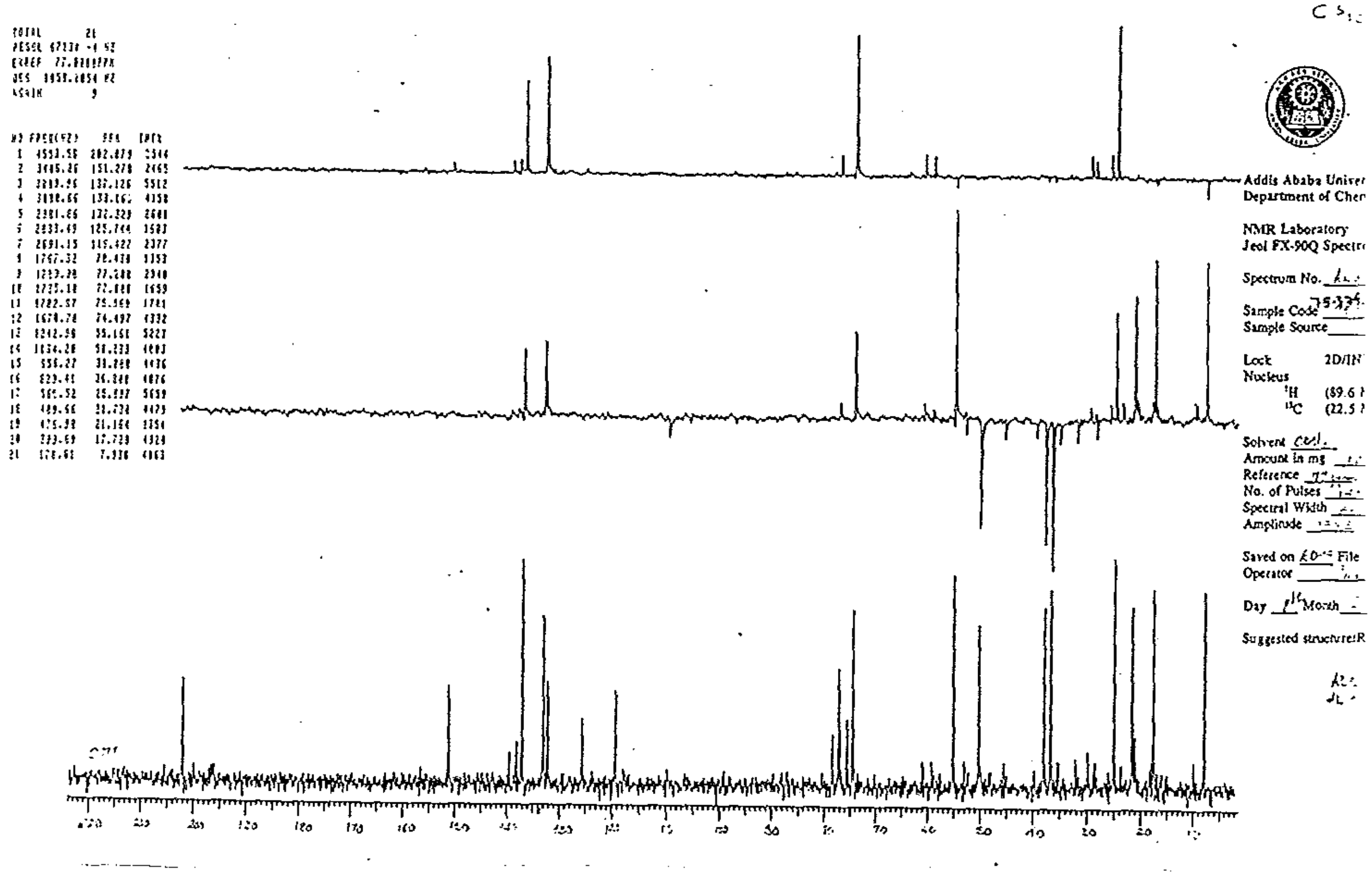
Day 18th Month April 199

Suggested structure/Remarks


Addis Ababa Univer
Department of Chen

NMR Laboratory
Jeol FX-90Q Spectr

Spectrum No.

Sample Code 75-37
Sample Source

Lock 2D/IN
Nucleus
^1H (89.6
^{13}C (22.5

Solvent $CDCl_3$
Amount in mg
Reference
No. of Pulses
Spectral Width
Amplitude

Saved on FD- File
Operator

Day 11th Month

Suggested structure/R

Fig 3.6 ^1H and ^{13}C NMR of Cs-10

Table 3.4. ^{13}C NMR data for compounds **Cs-6**, oxidized **Cs-6**, **cs-9** and **cs-10**

	Chemical shifts (δ_c)			
Carbon number	**Cs-6**	**Cs-6(oxidized)**	**Cs-9**	**Cs-10**
1	145.5 d	145.9 d	135.0 d	133.1 d
2	115.6 t	116.o t	132.0 d	74.5 d
3	112.9 t	113.4 t	77.8 d	36.8 t
4	165.5 s	165.5 s	37.9 d	25.1 d
5	64.1 d	64.5 d	48.0 t	50.3 t
6	77.2 d	194.9 s	203.0 s	202.1 s
7	119.2 s	119.2 s	117.2 s	119.4 s
8	141.0 s	141.0 s	151.0 s	151.3 s
9	33.6 t	34.0 t	33.3 t	38.4 t
10	42.8 s	42.8 s	142.1 s	132.3 s
11	141.0 s	137.7 s	128.1 s	125.7 s
12	139.5 d	139.9 d	137.4 d	137.1 d
13	8.8 q	9.4 q	8.6 q	7.9 q
14	24.7 q	25.2 q	18.4 q	21.7 q
15	24.7 q	22.6 q	115.0 t	17.7 q
OCH_3			56.1 q	55.1 q

4. EXPERIMENTAL

4.1 General

The essential oils from the plant materials were obtained by steam distillation at atmospheric pressure using 4 l round bottom flask fitted with Clavenger apparatus and a glass condenser. The oils were separated from the distillate and dried over anhydrous sodium sulphate. Neat optical rotations were measured using using P20 polarimeter. Specific gravity of the oils was determined using a pycnometer.

Analytical TLC were run on a 0.25 mm thick layer of silica gel GF_{254} (Merk). Components of the resins were detected by their UV fluorescence and 1% vanillin H_2SO_4 spray reagent. Flash Column Chromatography was performed using silicagel 60 (230-400 mesh) (Merck). Gas chromatographic analysis of the oil and standard samples was performed on Varian model 3700 gas chromatograph equiped with flame ionization detector (FID) using DB-5 fused silica Capillary column with 30 m x 0.23 mm internal diameter and 1.5 micron film thickness. Analysis were performed by programming the column temperature 60^0C to 200^0C at 4^0C/min. Injector and detector temperatures were 200^0C and 270^0C respectively. N_2 at 25 ml/min used as a carrier gas.

NMR spectra in $CDCl_3$ (^1H 90 MHz and ^{13}C NMR 22.5 MHz) were obtained on Jeol F x 90 Ω spectrophotometer. The NMR spectra (300 MHz for ^1H and 75 MHz for ^{13}C) were also recorded on a Bruker AMXR 300 NMR spectrometer with TMS as internal standard and δ values are given in ppm relative to TMS internal standard. The mass spectra were recorded at 70 eV using direct probe insertion.

4.2 Solvent systems

Different solvent systems were used for, thin layer chromatography (TLC), preparative thin layer chromatography (PTLC) and column chromatography (CC) see Table 4.1.

Table 4.1 Solvent systems used for TLC, PTLC and CC

No.	Solvent system	Ratio
I	Benzene-petrol-EtOAc	3:18:1
II	Petrol-EtOAc	19:1
III	Benzene-petrol-EtOAc	3:12:1
IV	Benzene-petrol-EtOAc	3:4:1
V	Petrol-EtOAc mixtures	

4.3 Plant materials

Leaves, fruits, bark and resin of *Boswellia neglecta* S. Moore locally known as "*Dakara*" (Oromo) were collected in Dec., 1996 and Mar. 1997 from Dibluk, Sidamo, 625 km S of Addis Ababa. It is tree *ca.*2.5 m tall. The plant was identified by K. Vollesen. Leaves, fruits, bark and resin of *C. sphaerocarpa* Chiov. were collected in Oct., 1996 and Jan. 1997 from Sof Omar, Bale, 105 km E of Robe. Its is tree *ca* 5 m tall. It was also identified by K. Vollesen.

4.4 Steam distillation and extraction of resins

The black crude resin of *B. neglecta* (300 g) was ground and steam distilled. The distillate was then dried with anydrous Na_2SO_4 and concd to yield a yellowish oil (13.6 g, 5%).

The resin of *C. sphaerocarpa* (100 g) was ground and steam distilled. Anhydrous Na_2SO_4 was added to the distillate and then concd in vacuo to yield a yellowish (2 g, 2%) oil.

The ground black resin of *B. neglecta* (100 g) was extracted twice with petrol

(200 ml) using sonic bath (15 min) and concd to give a yellowish extract (36 g, 36%). The residue was then extracted with EtOAc and concd under red. Pres. to yield (20 g, 20%). The remaining residue was subsequently extracted with MeOH to yield (7 g, 7%).

The crude resin of *C. sphaerocarpa* (20 g) was ground and extracted with petrol at room temp. for 1 day. The extract was filtered and concd to yield a yellowish extract (2.1 g, 11%). The residue was then extracted with EtOAc and MeOH when concd gave a brownish extract (2.5 g, 13%) and a brownish dark extract (1 g, 5%) respectively.

4.5 Isolation of compounds

Twenty-six fractions were collected when 15 g petrol fraction of the resin of *B. neglecta* was applied on CC over silica gel eluting with petrol/EtOAc gradients (solvent system V) and analysed by TLC. Fraction 18 (120 mg) was further purified by PTLC (solvent system I) yielded the major component **Bn-8** (78 mg). Compound **65** was isolated from the resinoid of *B. neglecta.*

12-ursen-3-ol (**65**): Colour white; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.01 (3H, s, Me), 1.24 (3H, s, Me), 1.37 (3H, s, Me), 1.41 (6H, m, 2 x Me), 3.64-3.74 (1H, m, 3-H), 5.36 (1H, m, 12-H); MS *m/z* (rel. int. %): 426.4 $[M]^+$ (12), 218.2 (100), 203.2 (16), 189.1 (11), 175.1 (6), 95.1 (8), 55.1 (6); ^{13}C NMR (75 MHz, $CDCl_3$): See Table 3.3.

The petrol extract of the resin of *C. sphaerocarpa* (2 g) was subjected to CC over silica gel and elution was carried out using petrol-EtOAc mixtures (solvent system V) of increasing polarities. A total of 33 fractions each 100 ml were collected and analysed by TLC. Fractions 4 and 5 (11 mg), fractions 12-14 (8 mg), fractions 17-19 (108 mg), fractions 26-29 (83 mg) and fractions 30-32 (238 mg) were combined. Fractions 4 and 5 afforded **Cs-4** (11 mg), fraction 20 yielded **Cs-6** (89 mg) and fractions 26-29 gave **Cs-9** (83 mg). Fractions 17-19 (36 mg) were combined and further purified by

PTLC (0.5 mm silica gel plate, solvent system III) displayed

3 bands. **Cs-5** (11 mg) was obtained from the first band.

Fifty-two fractions 100 ml each were collected when 2 g EtOAc fraction of the resin of *C. sphaerocarpa* was applied on CC over silica gel eluting with petrol-EtOAc gradients (solvent system V). The fractions were analysed by TLC. Fraction 6 (8 mg) and fraction 7 (2 mg) were combined to give **Cs-4** (10 mg). Combined fractions 16 and 17 (50 mg) were further purified by PTLC (solvent system II) yielded **Cs-5** (10 mg). Fractions 24 and 25 were combined and purified by PTLC (solvent system III) to collect 2 bands. Band II afforded **Cs-10** (15 mg).

The MeOH fraction of the resin of *C. sphaerocarpa* (20 g) was chromatographed on silica gel and eluted with Petrol/EtOAc gradient (solvent systemV). Twenty 22 fractions each 75 ml were collected. Fraction 9 was purified by PTLC (0.5 mm silica gel plate, solvent system II). The PTLC displayed 2 bands. **Cs-9** (40 mg) was obtained from the first band. Fraction 8 was further purified by PTLC (solvent system II) and 2 bands were collected. **Cs-5** (37 mg) and **Cs-6** (37 mg) were obtained from this fraction.

4.6 Ehrlich reaction of Cs-6, Cs-9 and Cs-10

One drop of Ehrlich reagent was added to two drops of soln of **Cs-6**, **Cs-9** and **Cs-10** in $CHCl_3$. The mixture was changed to a red violet after few seconds.

Soln of **Cs-6**, **Cs-9** and **Cs-10** in $CHCl_3$ were applied on a thin layer chromatography (TLC) and developed with solvent system III. The chromatogram was freed from the mobile phase in a current of air. The Ehrlich reagent was then sprayed and the chromatogram was again dried. Red coloured spots of **Cs-6**, **Cs-9** and **Cs-10** were observed.

4.7 Reaction of 2,4-dinitrophenylhrydrazine with Cs-9 and Cs-10

Soln of **Cs-9** and **Cs-10** in $CHCl_3$ were applied on a thin layer chromatography (TLC) (solvent system III). The chromatogram was then dried with a current of air and sprayed with 2,4-dinitrophenylhydrazine reagent. Yellow to orange-yellow chromatogram zones on an almost colourless background was observed after drying the chromatogram [56].

4.8 Oxidation of Cs-6 with Pyridinium Cholorochromate

To a suspension of 5 mg PCC (prepared as described below) in CH_2Cl_2 3 mg **Cs-6** dissolved in CH_2Cl_2 was added. The mixture was stirred at room temp. for 12 hr. The compound analysed by TLC using 2,4-dinitrophenylhydrazine as a spray reagent formed yellowish orange spot.

Preparation of the reagent:

2.5 g of chromium (vi) oxide was added with stirring to 4.5 ml 6 M HCl. After 5 min the soln was cooled to 0^{0}C. 2 g of pyridine was added with stirring to the solution in the period of 10 min. The pyridinium chlorochromate (PCC) crystallizes out as orange needles, was filtered by suction using sintered funnel and for 12 h under vacuum in a desicator with calcium chloride ($CaCl_2$).

4.9 Acetylation of Cs-6 with acetic anhydride

A mixt. of 3 mg **Cs-6**, 2 drops of pyridine and 1 ml of acetic anhydride was heated at 60-70^0 C for 6 hr. The soln was basified with 10% $NaHCO_3$ and extracted with dichloromethane. The organic layer was dried with anhydrous sodium sulphate. The solvent was then evaporated under red. pres. to give 4 mg of the acetylated product. The R_f value of the acetylated product was compared with that of **Cs-6**.

5. REFERENCES

1. Vollesen K. (1989). Burseraceae. In " Flora of Ethiopia", Vol. 3 (ed. I. Hedberg, and S. Edwards), National Herbarium, Addis Ababa University, Addis Ababa, pp. 442-478. 2. Howes, F.N. Sc, D. (1949). Vegetable gums and resins. The Chronica Botanica Company of Waltham, New York, Vol xx, pp. 149-153.

3. Coppen, J. J. W. (1995). Non-wood forest products 1: Flavours and fragrance of plant origin, FAO, Rome, pp. 81-92.

4. Bekele, A., Birnie, A. Tengnas B. (1993). Useful trees and shrubs for Ethiopia, Regional soil conservation unit, Swedish International Development Authority pp. 166-167.

5. Khalid, S.A. (1983). In "Chemistry and Chemical Taxonomy of the Rutales", Ed. Waterman, P.G. and Grundon M.K., Academic Press, London, pp. 281-297.

6. Maradufu, A. (1982). Fuanosesquiterpenoids of *Commiphora erythraea* and *C. m yrrh. Phythochemistry*, **21**, 677-680.

7. Guenther, E. (1950). The essential oils. Vol. 4. Van Nostrand, Princeton, NJ. pp. 331-356.

8. Tucker, A.O. (1986). Frankincense and myrrh. *Economic Botany*, **40**, 425-433.

9. Brieskorn, C.H. and Noble , P. (1980). Drei neue furanogermacrene aus murrhe. *Tetrahedron Lett.*, 1511-1514.

10. Brieskom, C.H. and Noble, P. (1982). Inhaltsstoffe des etherischen oils der myrrhe. *Planta Med.*, **44**, 87-92.

11. Brieskorn, C.H. and Noble, P. (1983). Two furanoeudesmanes from the essential oil of myrrh. *Phytochemistry*, **22**, 187-189.

12. Brieskorn, C.H. and Noble, P. (1983). Furanosesquiterpenes from the essential oil of myrrh. *Phytochemistry*, **22**, 1207-1211.

13. Provan, P.G., Gray, A.I. and Waterman, P.G. (1987). Sesquiterpenes from the myrrh-type resins from some Kenyan *Commiphora* species. *Flavour Fragr*. J., **2**, 109-113. 14. Thulin, M. and Claeson, P. (1991). The botanical origin of scented myrrh (Bissabol or habak hadi). *Economic Botany*, 45, 487-494.

15. Joule, J. A., Smith G. F. (1978). Heterocyclic Chemistry, 2nd ed. Van Nostrand Reinhold, London, pp.199-201.

16. Katritzky, A.R. and Lagowski, J.M. (1960). Heterocyclic Chemistry, Methuen & CO LTD, London, pp. 166-169.

17. Katritzky, A.R. and Lagowski, J.M. (1967). The principles of heterocyclic chemistry. Academic Press Inc., New York, pp. 112.

18. Gilchrist, T.L. (1992). Heterocyclic chemistry, Second ed., Longman, New York, pp. 195-196.

19. Mann, J. (1980). Secondary metabolism, Oxford Chemistry series, Clarendon Press, Oxford, pp. 79-116.

20. Mann, J., Davidson, R.S., Hobbs, J.B., Banthorpe, D.V. and Harborne, J.B. (1994). Natural products: Their Chemistry and biological significance, Longman scientific & Technical, New York, pp. 289-359.

21. Burchfield, H.P. and Storrs, E.E. (1962). Biochemical applications of gas chromatography, Academic press Inc., London, pp. 371-395.

22. Keulemans, A.I.M. (1959). Gas chromatography, 2nd edn. Reinhold Publishing Corp.,

New York, pp. 26.

23. Ettre, L.S. and McFadden, W.H. (1969). Ancillary techniques of gas chromatography. John Wiley & Sons, Inc., New York, pp. 146.

24. Patil, V.D., Nayak, U.R., and Dev, S. (1972). Chemistry of Ayurvedic crude drugs-I. Guggulu (resin from *C. mukul*)-1: Steroidal constituents. *Tetrahedron*, **28**, 2341-2352.

25. Kapoor, N. K. (1972). Hypocholesterolemic effect of *C. mukul* resin (guggul). *Indian J. Exp. Biol.*, **9**, 376-377. (*Chem. Abst.* **76** (1972) 41970d).

26. Malhotra, S. C., Ahuja, M. M. S. (1971). Comparative hypolipidemic effectiveness of gum guggul (*C. mukul*) fraction. *J. Med. Res.*, **59**, 1621-1632. (*Chem. Abst.*, **77**, (1972) 29208z).

27 Arora, R. B., Taneja, V., Sharma, R. C., Gupta, S. K. (1972). Antiinflammatory studies on a crystalline steroid isolated from *C. mukul*. *Indian J. Med. Res.*, **60**, 929-931. (*Chem. Abst.*, **77**, (1972) 160221r).

28. Arora, R.B., Kapoor, V., Gupta, S.K. and Sharma, R.C. (1971). Isolation of a crystalline steroidal compound from *Commiphora mukul* and its anti-inflammatory activity. *Indian J. Exp. Biol*, **9**, 403-404.

29. Arora, R. B., Kapoor, V., Gupta, S. K., Sharma, R. C. (1971). Isolation of a crystalline steroidal compound from *C. mukul* and its antiinflammatory activity. *Indian J. Exp. Biol.*, **9**, 403-404. (*Chem. Abst.*, **75** (1971) 143946x).

30. Patil, V.D., Nayak, U.R., and Dev, S. (1973). Chemistry of Ayurvedic crude drugs-II. Guggulu (resin from *C. mukul*)-2: Diterpenoid constituents. *Tetrahedron*, **29**, 341-348.

31. Ruecker, G. (1972). Monocylic diterpenes from the Indian gum guggul (*C. mukul*). *Arch. Pharm.*, **307**, 486-493. (*Chem. Abst.*, **77** (1972) 111554t).

32. Patil, V.D., Nayak, U.R., and Dev, S. (1973). Chemistry of Ayurvedic crude drugs-III. Guggulu (resin from *C. mukul*)-3 long-chain aliphatic tetrols, a new class of naturally occuring lipids. *Tetrahedron*, 29, 1595-1598.

33. Pachauri, S. P., Mukherjee, S. K. (1971). Evaluation of phagocytic index of rabbit reticuloendothelial system (RES) during hypocholesterolemic and antiatherogenic during administration. *Indian J. Physiol. Allied. Sci.*, **25**, 123. (*Chem. Abst.*, **79** (1973) 121809k).

34. Tripathi, S. N., Gupta, M., Sen, S. P., Udupa, K. N. (1975). Effect of a keto-steroid of *C. mukul* on hypercholetrolemia and hyperlipidemia induced by neomercazole and cholestrol mixiture in chicks. *Indian J. Exp. Biol.*, **13**, 15-18. (*Chem. Abst.*, **83**, (1975) 405z).

35. Tripathi, S. N., Gupta, M., Dwivedi, L. D., Udupa, K. N. (1974). Effect of a keto steroid of *C. mukul* on thyroid gland in laboratory animals. *Q. J. Surg. Sci.*, **10**, 158-163. (*Chem. Abst.*, **83**, (1975) 188662h).

36. Bhanotra, C. M. (1973). Pharmacologically active principle from *C. mukul* for use as blood lipid lowering agent. **Indian**, **138**, 525 (Cl. A61K27/00), 14 Feb. 1976, Appl. 149, 28 Apr. 1973, 2 pp. (*Chem. Abst.*, **92**, (1980) 11215z).

37. Mester, L., Mester, M. and Nityanand, S. (1979). Inhibition of platelet aggregation by “guggulu” steroids. *Planta Med.*, **37**, 367-369.

38. Bajaj, A.G. and Dev, S. (1982). Chemistry of Ayurvedic crude drugs-V. Guggulu (resin from *C. mukul*)-5: Some new steroidal components and stereochemistry of guggulsterol-I at C-20 and C-22. *Tetrahedron*, **38**, 2949-2954.

39. Craveiro, A., Corsano, S., Proietti, G. and Strappaghetti, G. (1983). Constituents of essential oil of *C. guidotti*. *Planta Med.*, **48**, 97-98.

40. Tripathi, Y.B., Malhotra, O.P., and Tripathi, S.N., (1984). Thyroid stimulating action of Z-guggulsterone obtained from *C. mukul*. *Planta Med.*, **50**, 78-80.

41. Waterman, P.G. and Ampofo, S. (1985). Dammarane triterpenes from the stem bark of *Commiphora dalzielii*. *Phytochemistry*, **24**, 2925-2928.

42. Provan, G.J. and Waterman, P.G. (1985). Picropolygamain: a new lignan from *Commiphora incisa* resin. *Planta Med.*, **51**, 271-272.

43. Provan, G.J. and Waterman, P.G. (1986). The Mansumbinanes: Octanordammaranes from the Resins of *Commiphora incisca*. *Phytochemistry*, **25**, 917-922.

44. Provan, G.J. and Waterman, P.G. (1988). Major Triterpenes from the resins of *Commiphora incisa* and *C. kua* and their potential chemotaxonomic significance. *Phytochemistry*, **27**, 3841-3843.

45. Wenninger, J. A., Yates, R. L. (1969). Constituents of opopanax oil: sesquiterpene hydrocarbons. *J. Ass. Offic. Anal. Chem.*, **52**, 1155-1161. (*Chem. Abst.*, **72**, (1970) 32055z).

46. Provan, G.J., Gray, A.I. and Waterman, P.G. (1987). Monoterpene-rich resins from some Kenyan Burseraceae, *Flavour Fragr. J.*, **2**, 115-118.

47. McDowell, P.G., Lwande, W., Deans, S.G. and Waterman, P.G. (1988). Volatile resin exudate from stem bark of *Commiphora rostrata*: Potential role in plant defence. *Phytochemistry*, **27**, 2519-2521.

48. Abegaz, B., Dagne, E., Bates, C. and Waterman, P.G. (1989). Monoterpene-rich resins

from two Ethiopian species of *Commiphora. Flavour Fragr. J.*, **4**, 99-101.

49. Fiurie, T.G and Snyckers, F.O. (1989). A pentacyclic triterpene with anti-inflammatory and analgesic acivity from the roots of *C. merkeri. J. Nat. Prod.*, **52**, 1129-1131.

50. Carroll, J.F., Maradufu, A. and Warthen, J.D. (1989). An extract of *C. erythraea* : a repellent and toxicant against *ticks.Entomol. exp. appl.*, **53**, 111-116.

51. Claeson, P., Andersson, R. and Samuelsson, G. (1991). T-Cadinol: a pharmacologically active constituent of scented myrrh: Introductory pharmacological characterization and high field ^{1}H- and ^{13}C-NMR data. *Planta Med.*, **57**, 352-356.

52. Claeson, P, Andersson, R., and Samuelsson, G. (1990). T-cadinol: A sesquiterpene with smooth muscle relaxing and antidiarrhoreal properties isolated from scented myrrh. *Planta Med.*, **56**, 685.

53. Provan, G.J., Gray, A.I. and Waterman, P.G. (1992). Mansumbinane derivatives from stem bark of *Commiphora kua. Phytochemistry*, **31**, 2065-2068.

54. Duwiejua, M., Zeitlin, I.J, Waterman, P.G., Chapman, J. Mhango, G.J. and Provan G.J. (1993). Anti-inflammatory activity of resins from some species of the plant family Burseraceae. *Planta Med.*, **59**, 12-16.

55. Cavanagh, I. S., Cole, M. D., Gibbons, S., Gray, A. I. and Waterman, P. G. (1993). A Novel sesquiterpene, 1,2-epoxyfurano-10(15)-germacren-6-one, from the resin of *C.holtziana* Engl. *Flavour Fragr. J.*, **8**, 39-41.

56. Funk, J., Wimmer, F. (1990). Thin layer chromatography. Reagents and detection methods. Fundamentals, Reagents I. Vol 1a, VCH verlagssesellschaft, MBH, Weinhelm, pp. 273-274.

57. Mahajan, B., Taneja, S. C., Sethi, V. K. and Dhar K.L. (1995). Two triterpenoids from *B. serrata* gum resin. *Phytochemistry*, **39**, 453-455.

58 Waller (1972). Biochemical applications of mass spectrometry, John Wiley & Sons, Inc., New York, pp. 372-373.

59 Buckingham, J. Donaghy, S. M. (1982). Dictionary of Organic compounds. Vol I, fifth ed., Chapman and Hall, New York, pp. 346.