In the Name of Allah,
the Most Beneficent,
the Most Merciful

PHYTOCHEMICAL INVESTIGATIONS ON SOME MEDICINAL PLANTS FROM THE FAMILIES EUPHORBIACEA AND LAMIACEAE

Thesis Submitted

for the Fulfillment of the Degree of

DOCTOR OF PHILOSOPHY

By

AMIR REZA JASSBI

International Centre for Chemical Sciences

H.E.J. Research Institute of Chemistry, University of Karachi,

KARACHI-75270, PAKISTAN

MARCH 1999

Dedicated to

My loving Parents,

Brother and Sisters

Acknowledgments

It gives me a great pleasure to express my heartfelt gratitude to my research supervisor Prof. Dr. Viqar uddin Ahmad, for his keen interest, valuable guidance and continuous encouragement throughout the course of my research work.

I am thankful to Prof. Dr. Abdulhossein Rustaiyan, Shahid Beheshti University, Tehran, Iran, my M.S. supervisor, with whom I began to do research on natural products. I would like to express my thanks to Prof. Dr. Masood Parvez for his help to determine the X-ray crystal structures of decipinone and decipinol ester A.

I can never forget the love and sincerity of my parents, brother and sisters, without their support it would be very difficult for me to finalize this work.

I would also like to express my deepest appreciation to (Late) Prof. Salimuzzaman Siddiqui, who founded H.E.J. Research Institute of Chemistry and Prof. Dr. Atta-ur-Rahman, to make it one of the finest research institute for natural products chemistry in the world.

I wish to express my sincere regards to Prof. Dr. Bina Shaheen Siddiqui, Prof Abdul Malik, Dr. M. Shaiq Ali and Dr. M. Iqbal Choudhary for their help, guidance and expert opinion during my research work.

I am very grateful to my colleagues and lab fellows for providing a friendly environment of work. Dr. F.V. Muhammad, Dr. M. Noorwala, Mrs. Fehmida T. Baqai, Mr. Naseer Alam, Mr. Murad Ali Khan, Mr. Mohammad Zahid, Mr. Mohammad Abbas, Mr. Zulfiqar Ali, Mr. Akbar Ali, Miss Shazia Yasmeen, Mrs. Farah Naz Zafar and Mr. S.R. Hussaini.

I am thankful to Dr. M. Shaiq Ali, Miss. Farah Mazhar, Mr. M Zahid, Miss S. Yasmeen and Mr. S.R. Hussaini for proof reading of my thesis and also special thanks to Mr. M. Rais Hussain for typing of thesis work. I wish to thank Mr. Rafat Ali for his patience and help throughout my research time and Mr. Mahmood Alam for his supports and help during my stay in H.E.J.

Finally I would like to express my acknowledgment to all of the technical and non-technical staff of H.E.J. Research Institute of Chemistry

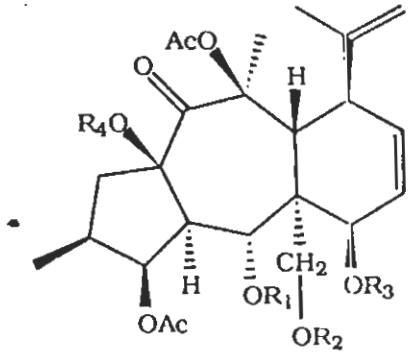
Amir Reza Jassbi
March 1999

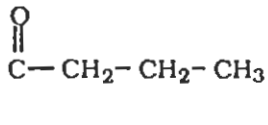
Table of Contents

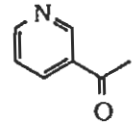
SUMMARY

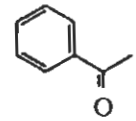
This dissertation describes the characterization of the chemical constituents of two plants of the genus *Euphorbia* from the family Euphorbiaceae, *Euphorbia decipiens* Boiss. & Buhse and *Euphorbia teheranica* Boiss., which are endemic to Iran, and the essential oil of *Zataria multiflora* Boiss. (Lamiaceae) from Pakistan.

Investigation of *E. decipiens* resulted in five novel diterpenoids with a new parent alcohol skeleton, named as decipinone (**79**), isodecipinone (**84**), decipidone (**85**), isodecipidone (**86**) and decipinone A (**87**), and two novel tetracyclic diterpenoids, decipinol ester A (**88**) and B (**90**). Karajinone A (**91**) and B (**94**) were identified as two new pentacyclic diterpene esters with a rare carbon skeleton related to lathyrol. The known compounds isolated from this plant included, β-sitosterol (**95**), cycloeucalenol (**96**), obtusifoliol (**97**), cycloart-23-ene-3,25-diol (**98**), and 24-methylenecycloartan-3β-ol (**99**)





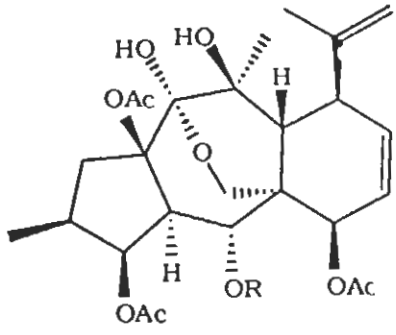




Bu=Butanoyl Nic=Nicotinoyl Bz=Benzoyl

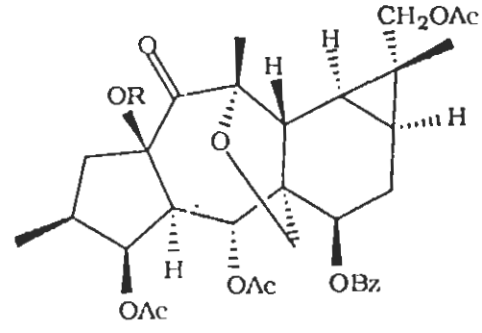
	R_1	R_2	R_3	R_4
Decipinone (**79**)	Bz	Ac	Ac	H
Isodecipinone (**84**)	Bz	H	Ac	Ac
Decipidone (**85**)	Bu	Ac	Ac	H
Isodecipidone (**86**)	Bu	H	Ac	Ac
Decipinone A (**87**)	Bu	Nic	Ac	H

(*Tetrahedron*, 1998, **54**, 1573-1584)
(*Planta Medica*, 1998, **64**, in press)



	R
Decipinol ester A (**88**)	Bz
Decipinol ester B (**90**)	Bu

(manuscript, under preparation)

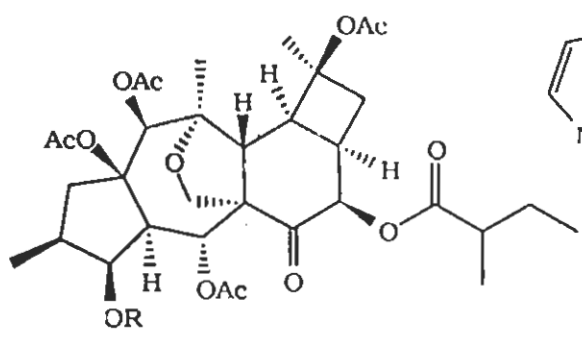


	R
Karajinone A (**91**)	Ac
Karajinone B (**94**)	H

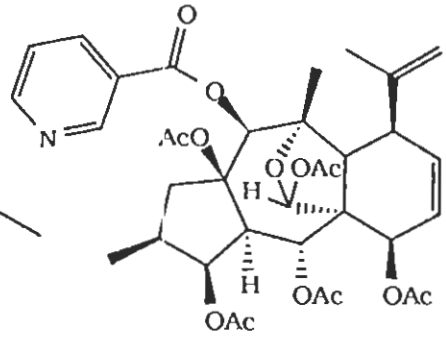
(*Phytochemistry*, 1998, **48**, 1217-1220)

Two new diterpene esters, tehranone A (**100**) and B (**101**) with a cyclomyrsinol skeleton and one diterpenoid, tehranol ester (**102**), related to the hydroxymyrsinol skeleton were identified as the chemical constituents of *E. teheranica*. A biogenetic pathway is suggested for the formation of the hemiacetal functionality of tehranol ester from decipinone skeleton rather than myrsinol.

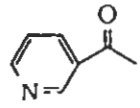
The known compounds identified in *E. teheranica* are: methyl gallate (**105**), betulin (**106**), erythrodiol (**107**), oleanolic acid (**108**) and β-sitosterol glycoside (**109**).



Tehranone A (**100**) R=Ac



Tehranol ester (**102**)

Tehranone B (**101**) R= 

(*J.Nat.Prod.*, manuscript submitted)

Analysis of the essential oil of *Zataria multiflora* Boiss. by means of GC, GC/MS and ^{13}C-NMR spectroscopy* resulted in qualitative and quantitative identification of twenty-four known compounds, including

---

* *J.Essent.Oil.Res.*, in press.

tricyclene (0.9%), α-thujene (2.0%), α-pinene (0.1%), camphene (0.4%), β-pinene (0.8%), myrcene (0.2%), *p*-cymene (7.1%), 1,8-cineol (0.5%), γ-terpinene (5.6%), terpinolene (0.4%), terpinene-4-ol (0.1%), α-terpineol (1.1%), carvone (0.1%), methyl carvacrol (6.5%), thymol (2.0%), carvacrol (62.4%), thymol acetate (0.6%), carvacrol acetate (4.4%), β-caryophyllene (0.6%), aromadendrene (0.8%), α-himachalene (0.2%) valencene (0.9%), 9-aristolen-1-α-ol (1.6%) and guaiol (0.2%).

1. INTRODUCTION

The genus *Euphorbia* is the largest in the plant family Euphorbiaceae. Among the different types of compounds found in the bioactive, skin irritant polycyclic diterpenoids of the tigliane and ingenane types are common in the milky latex (1).

Some of the plants belonging to the genus *Euphorbia* are used in folk medicine in order to cure some skin diseases gonorrhea, migraine, intestinal parasites and warts (2). *E. kansui* is considered as a herbal remedy for edema, ascites and cancer in China. Investigation showed two antileukemic diterpene esters with an ingenane carbon skeleton (3). Some esters of myrsinol, a tetracyclic diterpenoid isolated from *E. myrsinites* showed anti-HIV-1 reverse transcriptase (RT) inhibition (4).

The skin-irritant, tumor-promoting and anti-tumor, and recently anti-HIV activities of these plants prompted us to investigate two endemic plants of Iran, *Euphorbia decipiens* Boiss. & Buhse and *E. teheranica* Boiss. for their chemical constituents (5).

1.1 The Botanical Specifications of *Euphorbia*

The Euphorbiaceae or spurge family is one of the largest and most unwidely families of the angiosperms and because of the range of morphological variation it may be polyphyletic in origin (1). The genus

Euphobia is one of the six largest genera of flowering plants having some 2000 species, and is considered ± cosmopolitan, but is chiefly restricted to tropical, subtropical and warm temperate regions (6).

They are monoecious herbs, shrubs or trees, often succulent, with milky latex and with a simple indumentum, when present. Leaves are often of 3 types, lower, median and upper; lower or stem-leaves usually alternate, median or pseudumbel-leaves whorled, upper or ray-leaves whorled or opposite, free or connate; all or most leaves usually sessile, they are rarely shortly petiolate, stipulate or not, simple, entire or toothed, parni or palminerved. Stipules, when present, minute and subulate, interpetiolar and chaffy, glandular and sessile, or spiny. Inflorescence a cyathium, with 1 ♀ flower and several bracteate ♂ flowers enclosed in a gland-bearing involucre; cyathia axillary or arranged pseudodichasially, often in a 'pseudumbel' of radiating 'dichasia'. Involucre usually 5-lobed, with 1-5 glands alternating with them. Male flowers each consisting of a single stamen borne directly on its own pedicel; anther-cells subglobose, longitudinally dehiscent. Female flower consisting of a trilocular ovary on a pedicel which usually elongates in fruit, ovules 1 per cell; styles 3, free or connate at the base, stigmas often bifid. Fruits 3-celled, dehiscent into bivalved cocci; endocarp woody or cartilaginous. Seeds often carunculate; testa thin, crustaceous, smooth, ornamented or sculptured, albumen thick, embryo straight; cotyledons broad, flat (6).

1.2 The Biological Activities and Medicinal Uses of the Genus *Euphorbia*

The use of natural products in the treatment of various diseases has played an important role in medical therapy for many years, and plants of the genus *Euphorbia* are known to possess considerable medicinal and economical importance. They have been used for cure of cancers, tumours and warts for hundreds of years (7).

In this section, the application of some *Euphorbia* plants in traditional medicine and some of their biological activities will be described.

1.2.1 *E. acaulis*

The paste of rhizomes of *E. acaulis* has been used by some tribes of central India as a cure for inflammatory disorders (8).

1.2.2 *E. antisyphilitica*

The crude extract of latices of *E. antisyphilitica*, *E. royleana* and *E. lactea cristata* were found to be toxic to the aquatic snail *Indoplanorbis exustus* at a very low concentration (9). The antihepatotoxic activity of different fractions of *E. antisyphilitica* extract were screened in CCl_4, thioacetamide and galactosamine model employing an *in vitro* method of screening. Two water-insoluble compounds identified as ellagic and dimethyl ellagic acid are the active compounds in this plant (10).

This plant is a popular herbal remedy in India used for the cure of liver ailments (10).

1.2.3 *E. broteri*

The carbohydrate containing extract of this plant was converted into C_1-C_{10} hydrocarbons which have potential as fuels (11).

1.2.4 *E. calyptrata*

E. calyptrata is a poisonous shrub growing in the Sahara desert. A decoction obtained by natives from its roots is reputed to have some neurotoxic effects (12). The crude methanolic tincture extract from root and cell cultures of this plant were investigated and found to be active on the CNS (centeral nervous system). The bioassay guided fractionation of this plant yielded toxic helioscopinolides A and D (13).

1.2.5 *E. cyparissias*

Phytochemical investigations showed the presence of irritant diterpene esters and hopenone-B in this plant (14). Pharmacological studies suggested this plant has tumor-promoting activity in mouse skin *in vivo* (15).

1.2.6 *E. characias*

E. characias is widespread in the Mediterranean countries and is known to have a skin-irritant effect and causes blistering of human skin.

Phytochemcial and pharmacological investigation on the latex of this plant yielded some skin-irritant lathyrane diterpenoids (16,17).

1.2.7 *E. confinalis var. rhodesiaca.*

From the latex of the plant one skin irritant diterpenoid ester, 12-deoxyphorbol-16-isobutyrate-13-angelate was isolated. This plant (whole) is widely used in Zimbabwe in traditional medicine (18).

1.2.8 *E. deightonii* and *E. desmondi*

They are used in traditional medicine and for fencing, as arrow poisons in Nigeria. These two plants contain ingenol type diterpenoids (19).

1.2.9 *E. esula*

E. esula, or leafy spurge, is distributed worldwide and contains a skin-irritant, toxic, milky latex. The extracts of the plant have been used in folk medicine to treat various cancers, swellings and warts. The phytochemical and pharmacological studies confirmed the proinflammatory, tumor-promoting and anti-tumor activities of the plant extracts (20). Phytochemical investigation showed that ingenane type diterpenoids were responsible for these activities (20). This plant is also reported to be toxic to cattle, allelopathic to desired forage species, and has seriously impacted open-range livestock production in the upper great plains states of the U.S.A. (21, 22).

1.2.10 *E. ebracteolata*

Two phenolic compounds: 2,4-dihydroxy-6-methoxy-3-methyl-acetophenone and 2-hydroxy-6-methoxy-3-methylacetophenone-4-β-glucoside were isolated from roots of this plant which inhibited the growth of tuberculosis bacilli (23).

1.2.11 *E. fischeriana*

The extract of this plant is used in manufacturing of an ointment for psoriasis (A chronic skin disease in which red scaly popules and patches appear) (24).

1.2.12 *E. hermentiana*

The ornamental succulent plant, *E. hermentiana*, which is indigenous to southwest Africa and sold in plant stores in U.S.A., is skin-irritant for humans. From a dermatitis-producing acetone extract of this plant, five ingenane and six ingol diterpene esters were isolated (25).

1.2.13 *E. hirta*

This plant, which is native to Australia, has been widely used in traditional medicine for cure of various diseases, and has sedative, antipyretic and anti-inflammatory properties. The above activities were confirmed scientifically and the aqueous extract of the plant exhibited sedative anxiolytic properties in mice (26). Further investigation on the extract of this plant showed analgesic activity in mice.

antipyretic in rats and finally anti-inflammatory effect on an acute inflammatory process (26). *E. hirta* is also used as an antidiarrh-t-: plant (27).

1.2.14 *E. kansui*

The roots of *E. kansui* are used in Chinese folk medicine for the treatment of edema, ascites, and cancer (20). The ethanolic extract of the roots of this plant showed significant *in vitro* cytotoxicity (P-388) and *in vivo* antileukaemic activity against P-388 lymphocytic leukaemia in mice. The ingenol diterpenoids of *E. kansui* are considered as the biologically active components of this plant. Also the analgesic and anti-writhing agents kansuinin-A, kansuinin-B, 20-deoxyingenol-3-benzoate, 20-deoxyingenol-5-benzoate, ingenol-3-(2,4-decadienoate)-20-acetate, and 13-oxyingenol-13-dodecanoate-20-hexanoate were identified in this plant (3, 28).

1.2.15 *E. micractina*

This plant is usually found at high altitude in western China, and in Chinese folk medicine, it is used as an anti-tumor agent and wart remover (29).

1.2.16 *E. milii*

The latex of *E. milii* var. *hislopii* (Crown-of-Thorns) is a potent plant molluscicide that could be used against the snails which are intermediate hosts of *Schistosoma trematodes*, but investigations

showed the presence of tumor-promoting substances, which may be hazardous for people who use this plant continuously (30,31). The cultured cells of *E. milii* produced high amount of a red coloring material belonging to anthocyanins which were used for dyeing of textiles including wool, cotton and silk at low pH (32).

1.2.17 *E. lathyris*

E. lathyris produces the diterpene esters of lathyrol and ingenol, which are considered as carcinogenic and skin-irritant components, this plant can also be considered as a source for oleic acid (33).

1.2.18 *E. lagascae*

E. lagascae is a plant native to Spain. Investigation showed that it contains a large amount of oil in its seed (50% of the seed's weight). About two-thirds of the oil is vernolic acid (*cis*-12,13-epoxy oleic acid) which has several valuable applications for the oleochemical industry (34).

1.2.19 *E. lunulata*

The spurge, *E. lunulata* has been applied as a growth controlling agent for watermelon (35).

1.2.20 *E. lateriflora*

E. lateriflora is used in Nigeria as a natural drug in some traditional medicinal preparations. Its latex is used as a cure for ringworm, and in dilute aqueous solution as a purgative (36).

1.2.21 *E. myrsinites*

The plant contains a skin irritant latex which causes severe irritation of the skin and eyes during its collection. Two of its myrsinol type diterpene esters showed moderate anti HIV-1 reverse transcriptase (RT) inhibition (4).

1.2.22 *E. nivulia*

E. nivulia is used in traditional medicine to cure bronchitis and rheumatism (37). It is considered as a remedy for enlargement of the liver and spleen, syphilis, dropsy, general anasarca, leprosy, whooping cough, dyspepsia, jaundice and colic (38).

1.2.23 *E. peplus*

This plant is used in folk medicine to cure asthma, catarrh and warts (39).

1.2.24 *E. prostrata*

E. prostrata is applied as anti-inflammatory and blood purifier as folk medicine in India (40).

1.2.25 *E. pseudocactus*

This plant is reported to have an antimicrobial, antimalarial and antitumor activities (41).

1.2.26 *E. piscatoria*

E. piscatoria is a shrub native to the Maderia archipelago. It has a large amount of latex which has an irritant effect on the skin and mucous membranes. It is used by fisherman for fishing, because of its paralyzing action (42).

1.2.27 *E. poisonii*

E. poisonii has a latex which is highly irritant to human skin and if it comes in contact with the eye it causes blindness. In Nigeria the latex is used as a pesticide on millet farms. The pharmacological studies showed its latex components as both procarcinogenic and antitumor agents (43, 44).

1.2.28 *E. portulacoides*

E. portulacoides, a native plant of Chile, has been used as a purgative in the folk medicine. Its properties are similar to *E. lathyris* (45).

1.2.29 *E. prolifera*

This plant is indigenous to Yunnan province in China and is used in folk medicine for treatment of inflammation and tumors (46).

1.2.30 *E. quinquecostata*

It is a tree found in certain parts of Tanzania and Kenya. According to traditional healers in Tanzania, a preparation of the stem wood of this plant is used to accelerate wound healing and to treat stomach pains. Bioassay-guided purification of an EtOAc-soluble portion of the methanolic extract of the plant, which exhibited significant inhibition in a phorbol dibutyrate receptor-binding bioassay system, led to the isolation of four active compounds, two with an ingenane skeleton (47).

1.2.31 *E. splendens*

The latex of *E. splendens* var. *hislopii* has a molluscicidal action at low concentration against the vector snails of schistosomiasis (48).

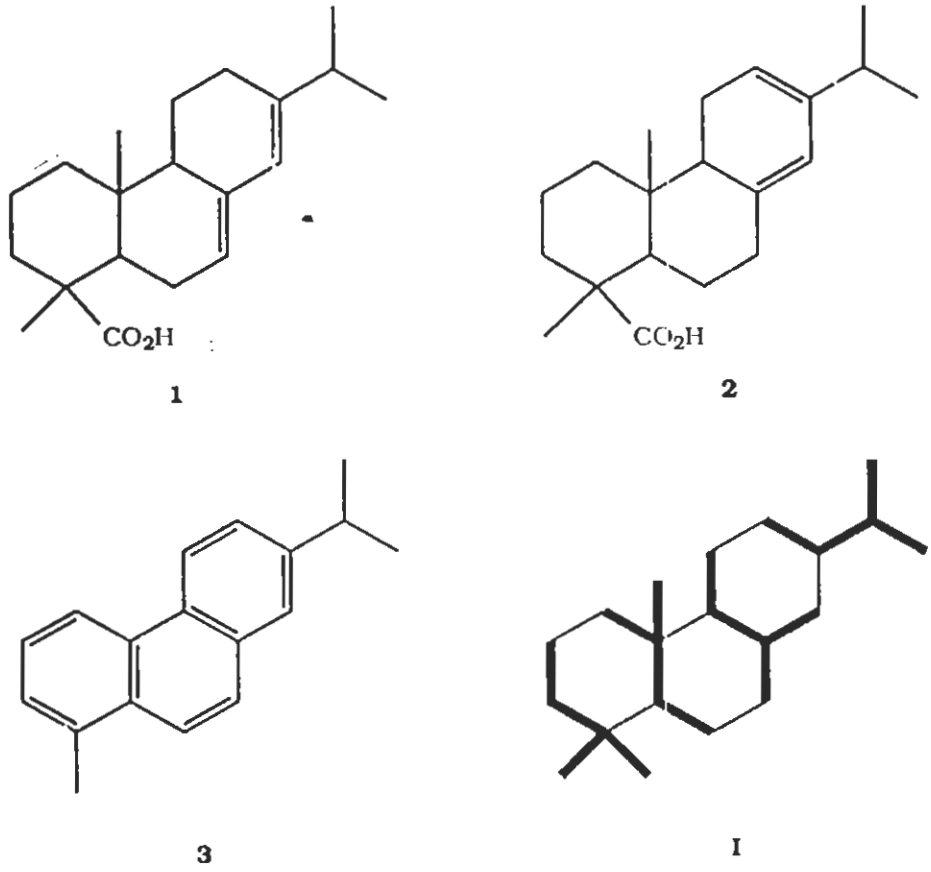
1.2.32 *E. tirucalli*

The anaerobic fermentation of this plant led to the production of biogas which contains 65% methane. The biogas was used in a generator for the production of electricity (49).

1.3 Classification of Diterpenoids

Diterpenoids are generally C_{20} compounds consisting of four isoprene (C_5H_8) units, however there are several naturally occurring compounds consisting either fewer, or more carbon atoms than 20, but because of their biosynthetic or historical relation with diterpenoids, they are considered in this category. The general name diterpenoids is preferred and used for diterpene (50).

The first diterpenoid was isolated from rosin (colophony) named as abietic acid. The resin acids are a mixture difficult to separate diterpenoids and in fact abietic acid (**1**) was separated in an impure form in 1824 and only in 1910 it was obtained as a pure compound. For the determination of the structure of these compounds, Ruzicka suggested two important ways, one dehydrogenation of diterpenes to known compounds, for instance dehydrogenation of abietic acid (**1**) and levopimaric acid (**2**) to retene (**3**), and secondly the isoprene rule, according to which the terpenoids are divisible to isoprene units, so the carbon skeleton (**I**) could be determined according to this rule (Fig. 1.1) (50-52).

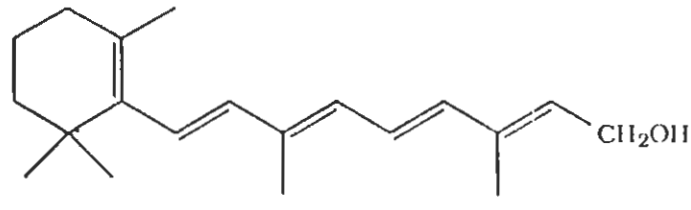


1 2

3 I

Figure 1.1: Application of dehydrogenation and isoprene rule for structure elucidation of diterpenoids by Ruzicka.

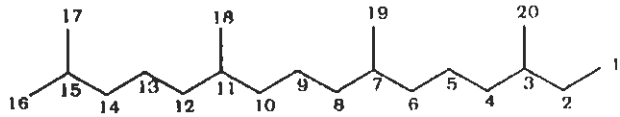
The structure of vitamin A (4) was also determined using isoprene rule by Karrer et al. (53).



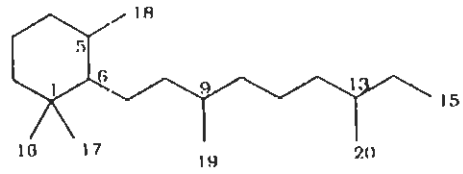
4

1.4 Structural Types of Diterpenoids

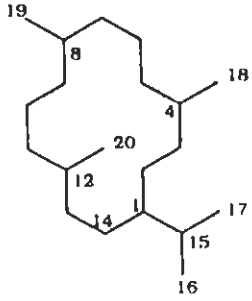
Progresses in the field of natural product chemistry caused by advances in spectroscopic and chromatographic techniques, and in the theory of organic chemistry, made the structure elucidation of natural products easier. So the number of known diterpenoids, which were about 650 by the end of 1970, reached to about 2000 compounds including more than 170 skeleta in the late of 1990's. These skeleta are related to some main skeleton divided according to their number of rings: Acyclic and monocyclic: [phytane (**5**), retinane or 10,15-cyclophytane (**6**) and cembrane (**7**)] bicyclic: [labdane (**8**), clerodane (**9**) and chettaphanane (**10**)], tricyclic: [lathyrane (**11**), fujinane (**12**), pimarane (**13**), rosane (**14**), abietane (**15**), totarane (**16**), cassane (**17**) and taxane (**18**), and finally tetra- and pentacyclic diterpenoids: [tigliane (**19**), gibberellane (**20**), andromedane (**21**), kaurane (**22**), beyerane (**23**), atisane (**24**), trachylobane (**25**) and aconane (**26**)] (Fig. 1.2) (50).



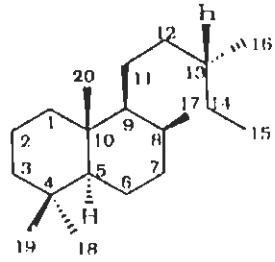
5



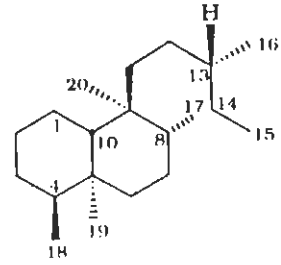
6



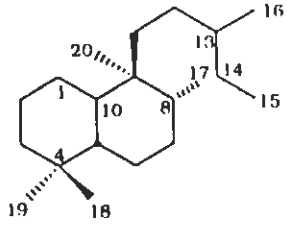
7



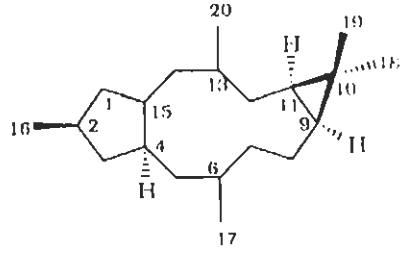
8



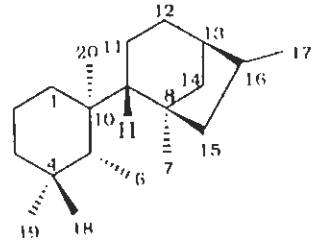
9



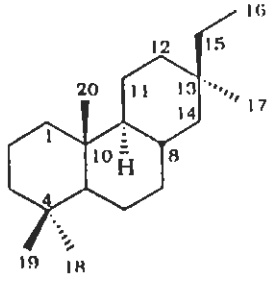
10



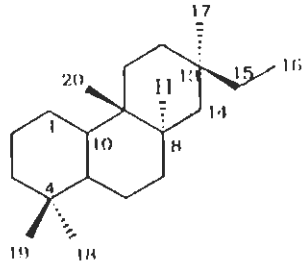
11



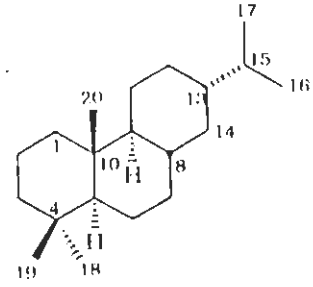
12



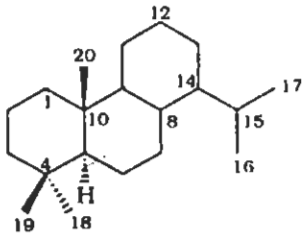
13



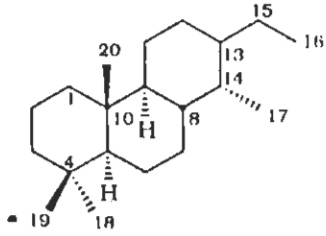
14



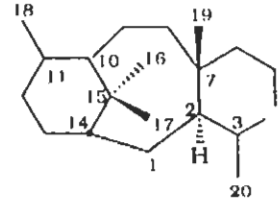
15



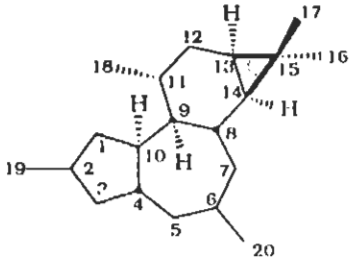
16



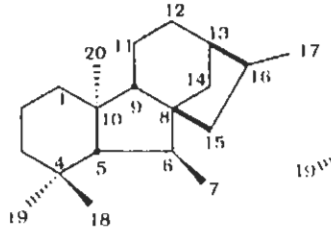
17



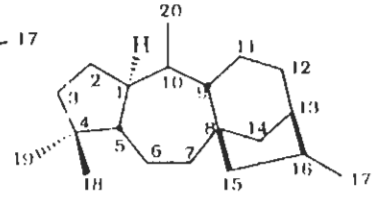
18



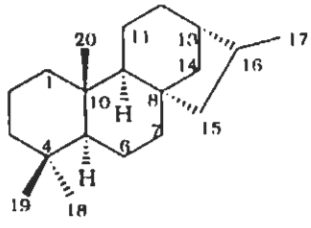
19



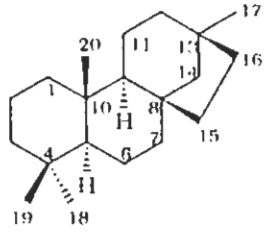
20



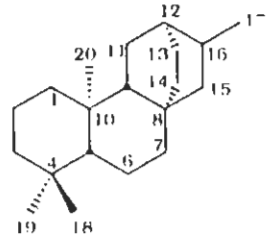
21



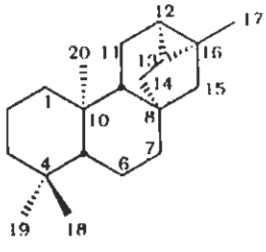
22



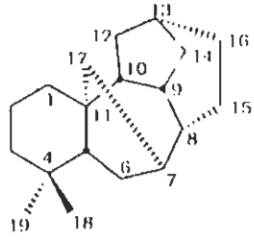
23



24



25



26

Figure 1.2: The main skeleta of diterpenoid according to the number of the rings.

The families Euphorbiaceae and Thymelacaceae are known to be rich in toxic poly- and macrocyclic diterpenes related to tigliane (**19**).

daphnane (**27**) and ingenane (**28**). One of the most important of these compounds is phorbol,which belongs to tigliane skeleton. The plants of family Euphorbiaceae possess some toxic, mostly macrocyclic diterpenoids which are related to hydrocarbon casbane (**29**). These compounds included lathyrane (**11**), jatrophane-A (**30**), jatrophane-B (**31**), crotofolane (**32**), jatropholane (**33**) and rhamnofolane (**34**). Recently some non-irritant polycyclic diterpenes with myrsinol type skeleton (**35**) related to lathyrane have been isolated from different species of *Euphorbia* plants (54).

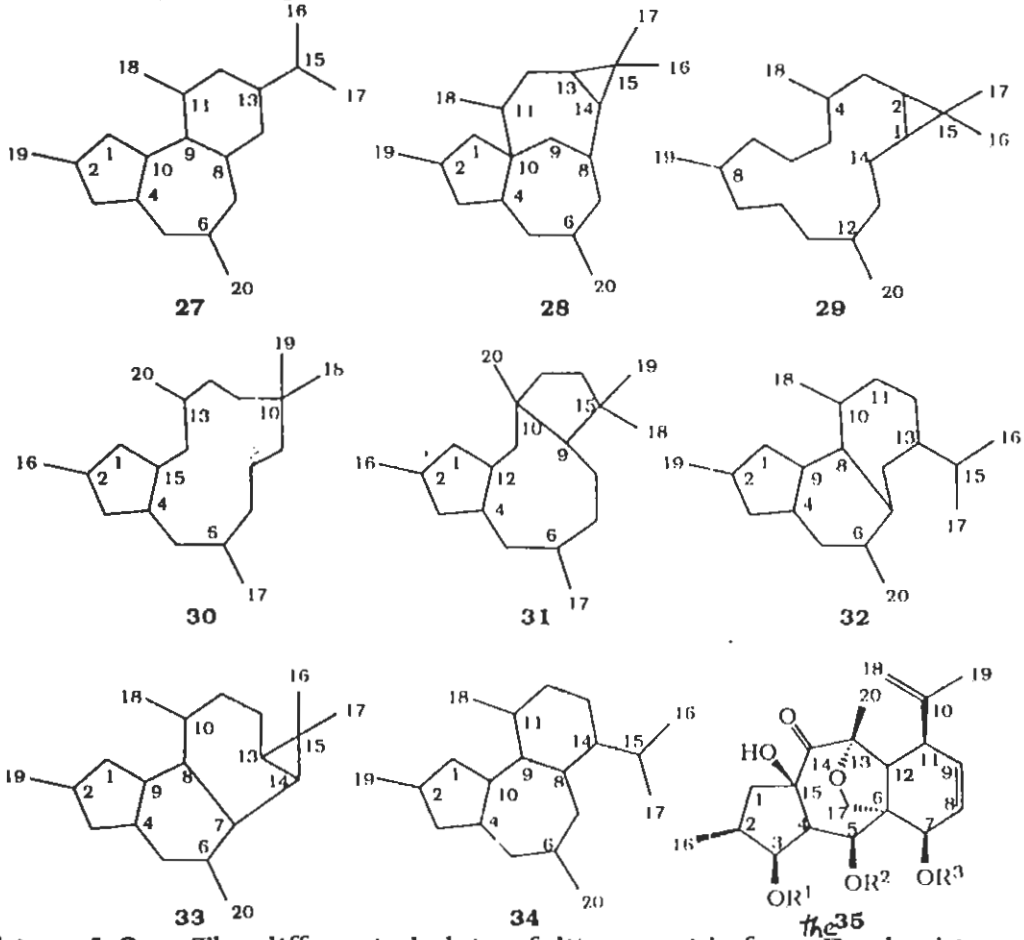


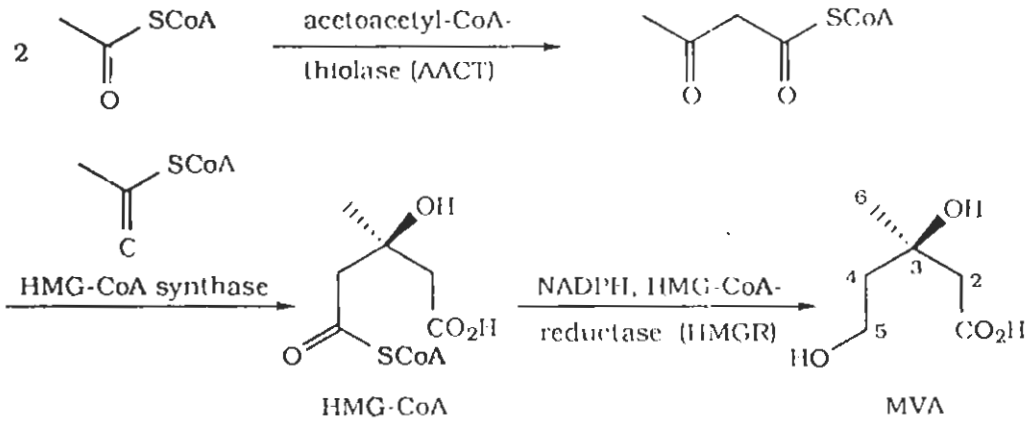
Figure 1.3: The different skeleta of diterpenoids from Euphorbiaceae and Thymelaeaceae.

1.5 Biosynthesis of Terpenoids

1.5.1 The Mevalonic Acid

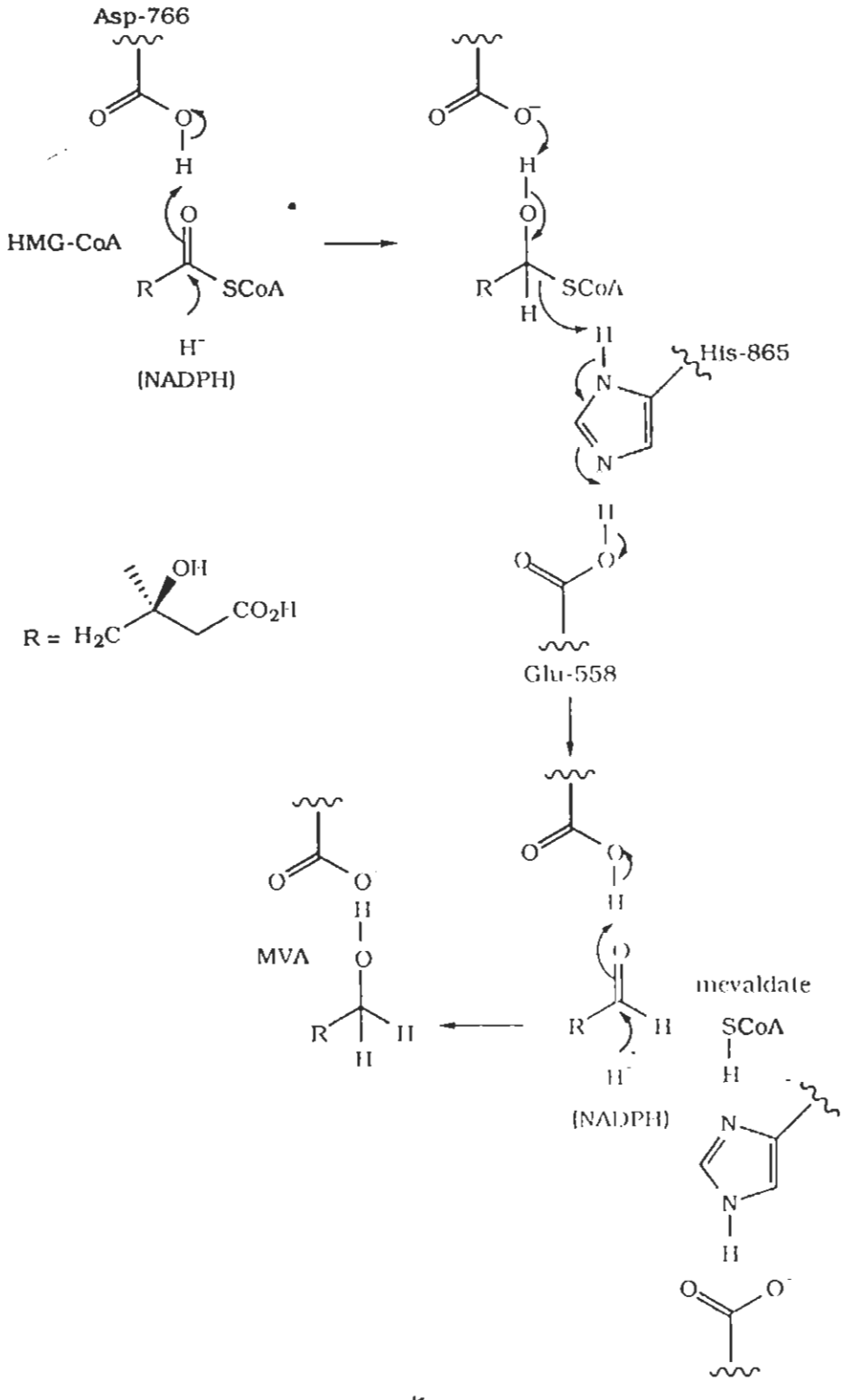
The biosynthesis of almost all isoprenoids begins from mevalonic acid (MVA) which is synthesized by the condensation of two molecules of acetyl-CoA to produce an acetoacetyl-CoA. Further aldol type condensation of another molecule of acetyl-CoA with acetoacetyl-CoA yields 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) which is catalyzed by HMG-CoA synthase enzyme.

HMG-CoA is then reduced to mevalonic acid (MVA) by HMG-CoA reductase (HMGR) which catalysed the NADPH (55).



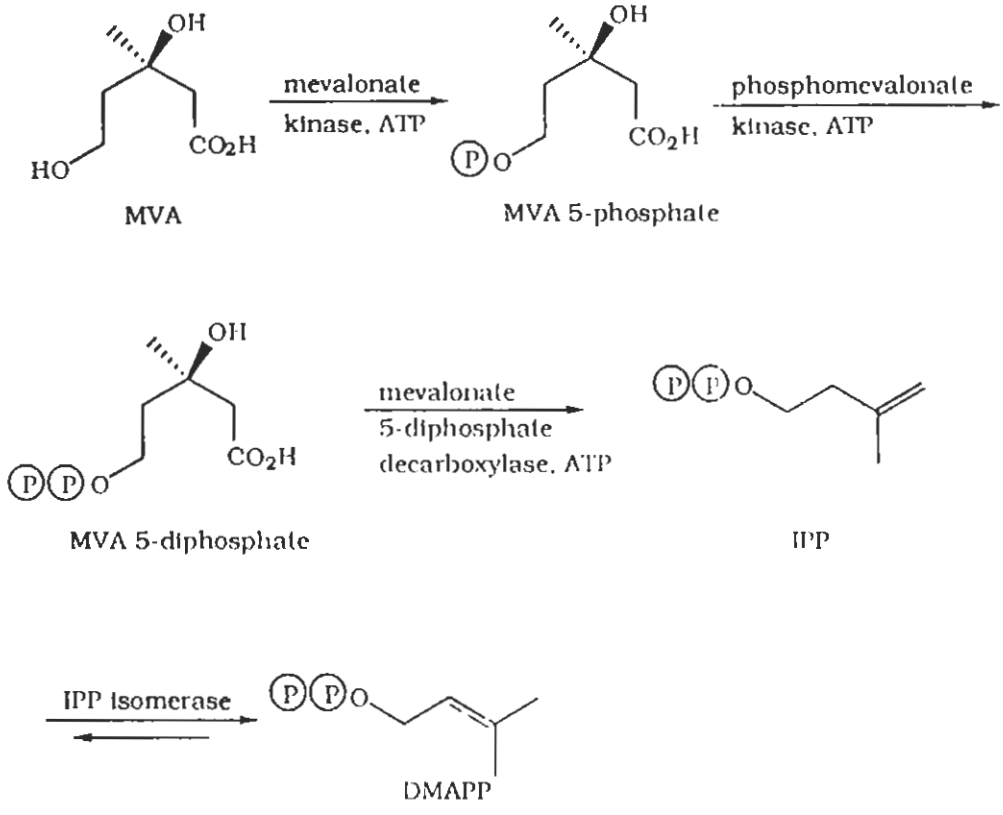
Scheme-1.1: Biosynthesis of mevalonic acid.

The suggested mechanism for the reduction of HMG-CoA to MVA is given in Scheme-1.2.



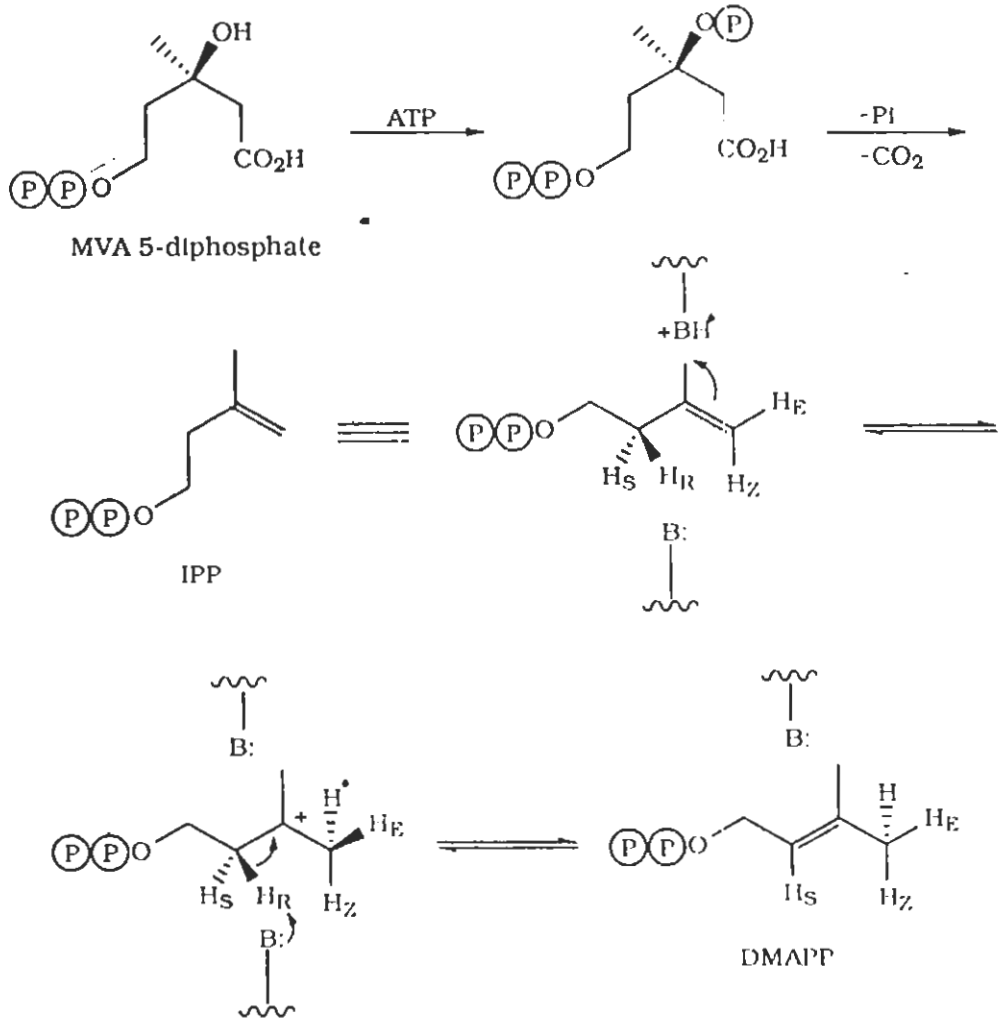
Scheme-1.2: The mechanism of the enzyme-catalysed reduction of HMG-CoA to MVA.

ATP-dependent phosphorylation of mevalonate to mevalonate-5-diphosphate followed by enzymatic decarboxylation yields isopentenyl diphosphate (IPP) which can be converted to dimethylallyl diphosphate (DMAPP) (Scheme-1.3).



Scheme-1.3: Conversion of MVA to DMAPP and IPP.

The mechanism of decarboxylation of MVA-5-diphosphate involves two steps, the phosphorylation of the 3-hydroxy group followed by decarboxylation (Scheme-1.4). The conversion of IPP to DMAPP is catalysed by IPP isomerase through a 1,3-allylic rearrangement through a postulated two base cationic mechanism (Scheme-1.4).

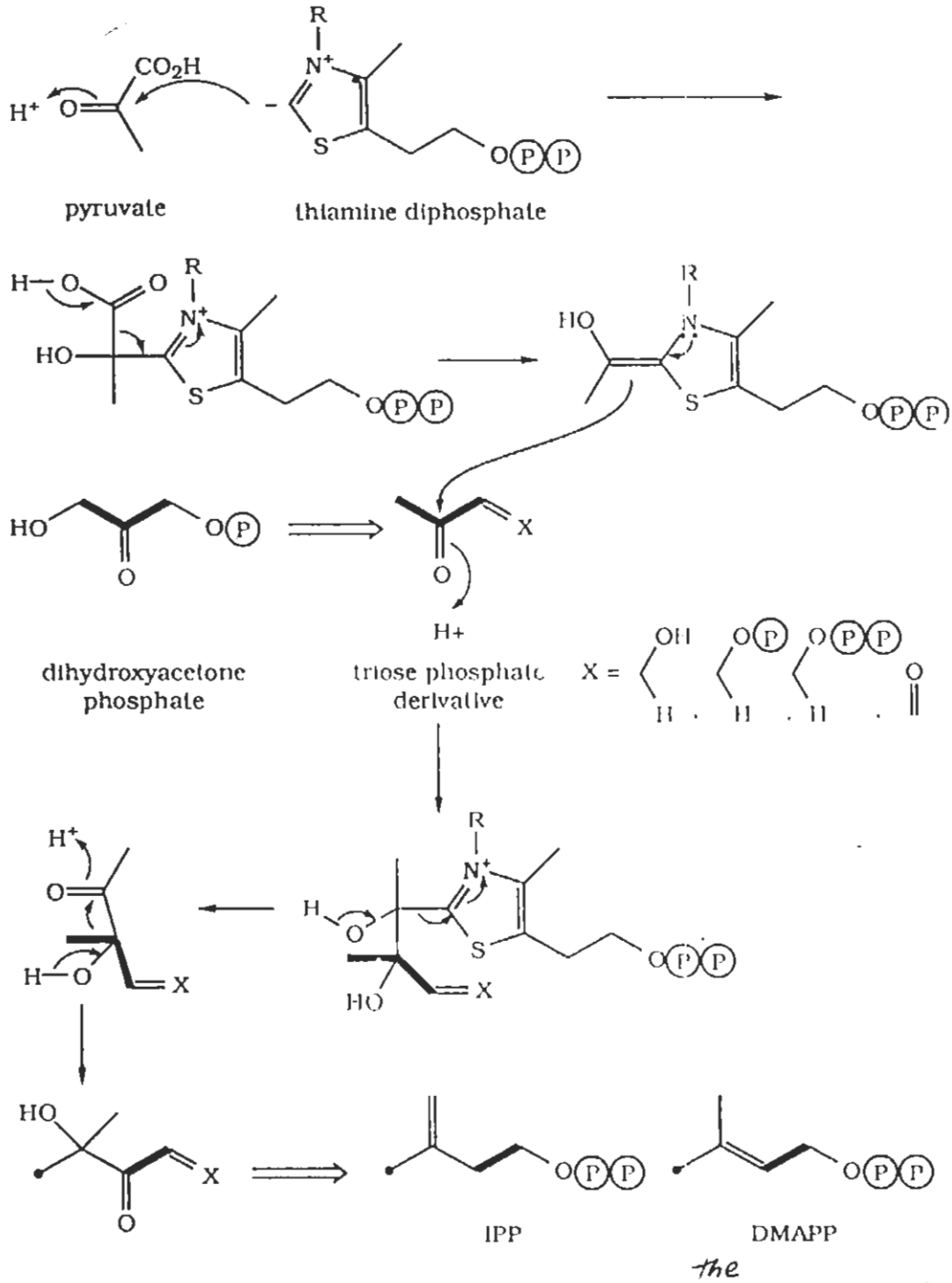


Scheme-1.4: Decarboxylation of MVA 5-diphosphate and isomerization of IPP to DMAPP

1.5.2 The Dihydroxyacetone phosphate pathway

A number of studies showed that some bacteria employ a biosynthetic pathway to terpenoids which is different from the mevalonate pathway. In this pathway the C_5 framework of terpenoids is considered to be constructed by the condensation of a C_2 unit derived from the decarboxylation of pyruvate onto the C-2 carboxyl of a triose phosphate derivative.

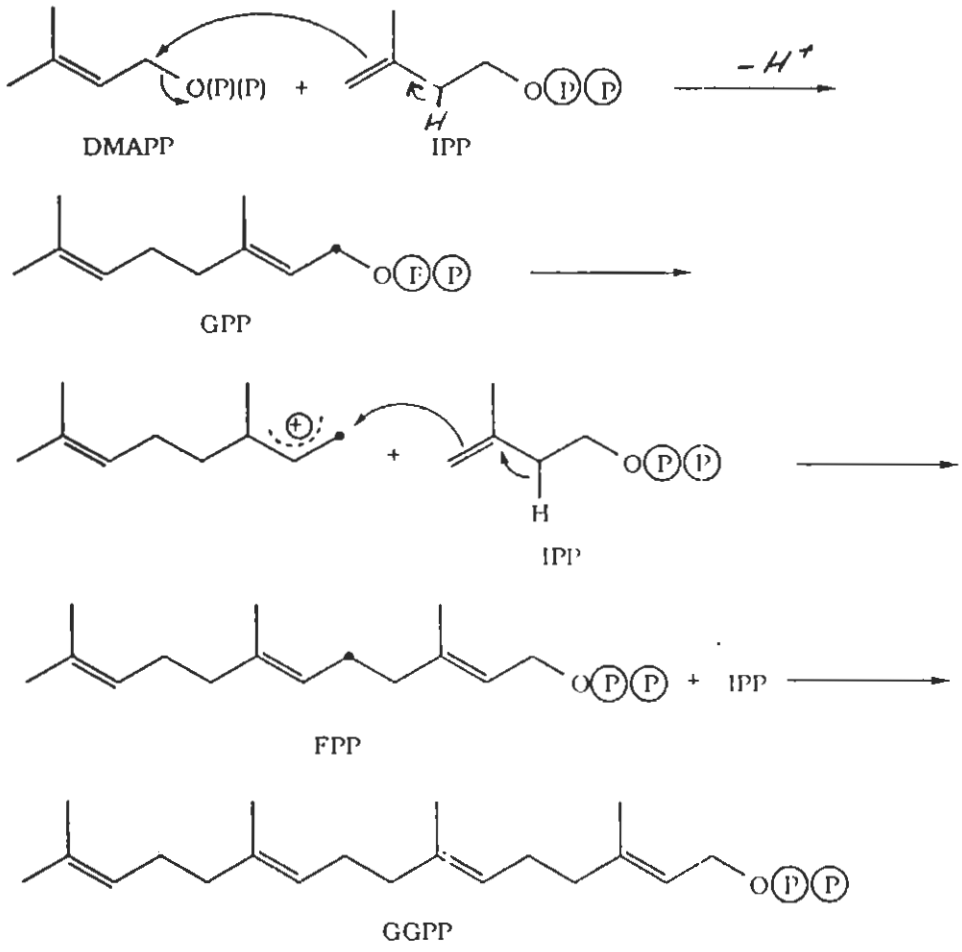
Scheme-1.5 illustrates the dihydroxyacctone phosphate pathway to produce IPP and DMAPP (55).



Scheme-1.5: The dihydroxyacetone pathway for the biosynthesis of IPP and DMAPP.

1.5.3 Alkylation Step in Terpenoid Synthesis

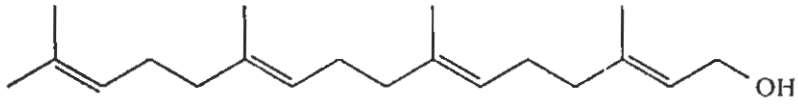
The enzymes known as the prenyltransferases are responsible for the alkylation steps involving DMAPP and one or more units of IPP. This reaction provides the precursors for terpenoids synthesis. The enzymes geranyl diphosphate synthase, farnesyl diphosphate synthase and geranylgeranyl diphosphate synthase are the enzymes which produce the precursors of mono-, sesqui- and diterpenoids, respectively, Scheme-1.6 (51, 55).



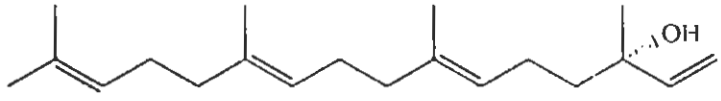
Scheme-1.6: Biosynthesis of GPP, FPP and GGPP from DMAPP and IPP.

1.6 Biosynthesis of Diterpenoids

As it was mentioned earlier, almost all diterpenoids are derived via cyclization of geranyl geraniol (**36**) or geranyl-linalool (**37**), either from a free radical or cationic pathway. This biogenetic isoprene rule was first suggested by L. Ruzica in 1953 (51).



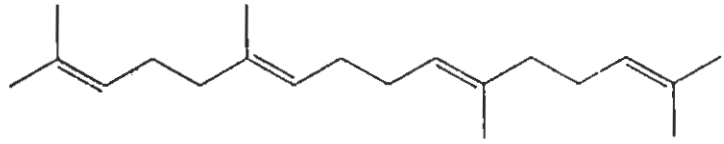
(36)



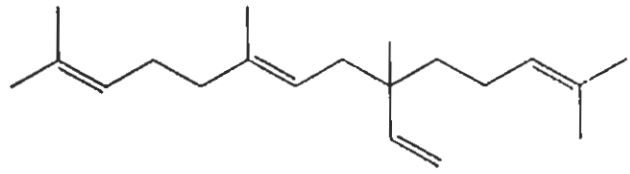
(37)

1.6.1 Acyclic Diterpenoids

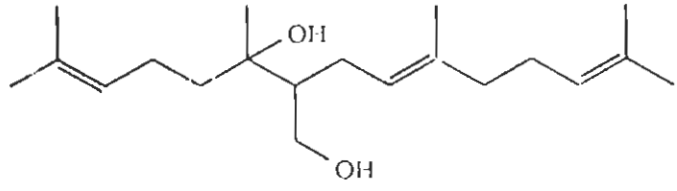
The acyclic diterpenoids with phytane (**5**) framework are the most important linear diterpenes, but, despite the fact that it is derived from geranyl-geranyl PP, the precursors of all diterpenes, the occurrence of related compounds are very rare. Some acyclic diterpenes are derived from precursors other than GGPP such as digeranyl (**38**) and isodigeranyl (**39**) and peucelinendiol (**40**) which are named as non-geranyl-geranyl diterpenoids (50, 56).



(38)



(39)

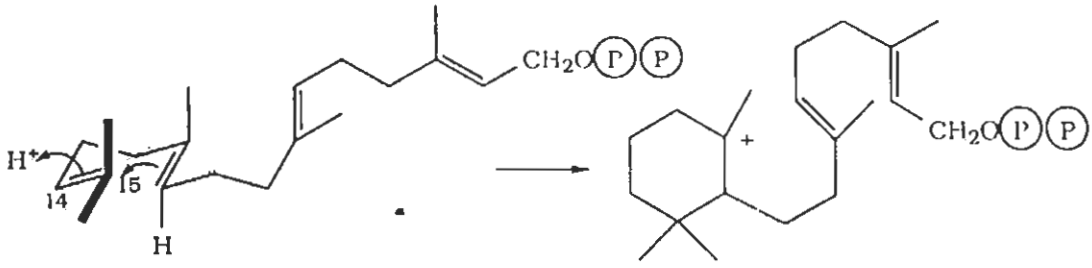


(40)

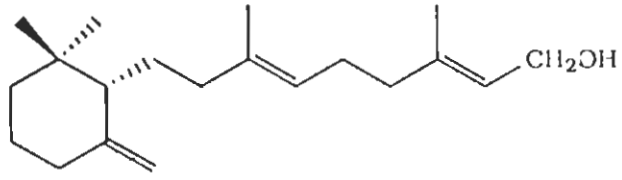
1.6.2 Monocyclic Diterpenoids

The biogenesis of these compounds involves the cyclization of GGPP. The biogenesis of the retinane type skeleton (**41**) and trixagol (**42**) can begin with the electrophilic attack of H^+ to the C-14/C-15 double bond of GGPP followed by dehyrogenation (Scheme-1.7).

The most important compound of this group, retinol is produced in the body by cleavage of certain carctonoid s. Trixagol and related compounds are apparently synthesized from the cyclization of GGPP (50,57).



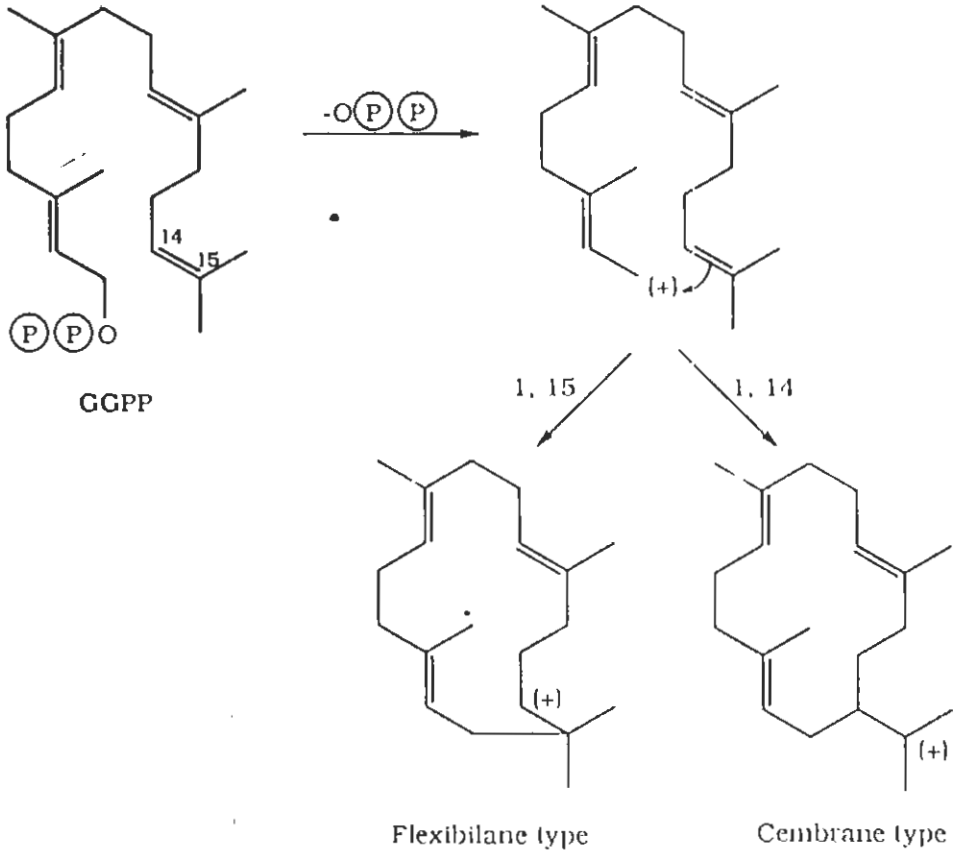
GGPP (41)



(42)

Scheme-1.7: Cyclization of GGPP to retinane.

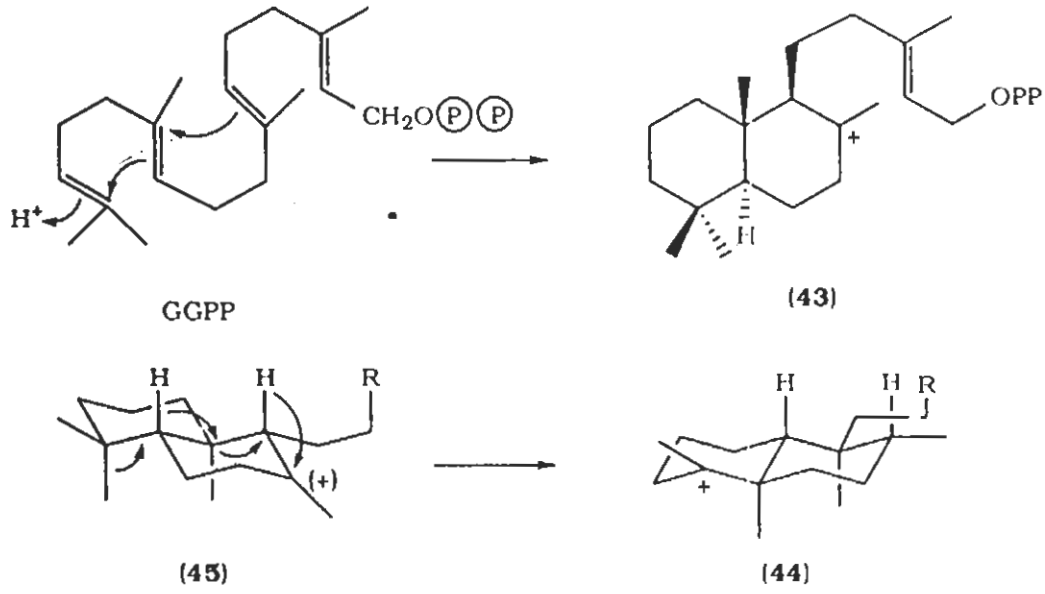
The intramolecular electrophilic attack of the C-14/C-15 double bond to the ionized GGPP at C-1 produces the macrocyclic diterpene, cembrene, which is widely distributed in resins, tobacco and marine species (58, 59). Although several diterpenoids with the cembrane skeleton have been isolated, flexibilene, a metabolite of the soft coral, *Sinularia flexibilis*, is a rare diterpenoid with a 15 membered ring resulting from the C-1/C-15 cyclization of GGPP (60) (Scheme-1.8).



Scheme-1.8: Biogenesis of macrocyclic diterpenoids from GGPP.

1.6.3 Bicyclic Diterpenoids

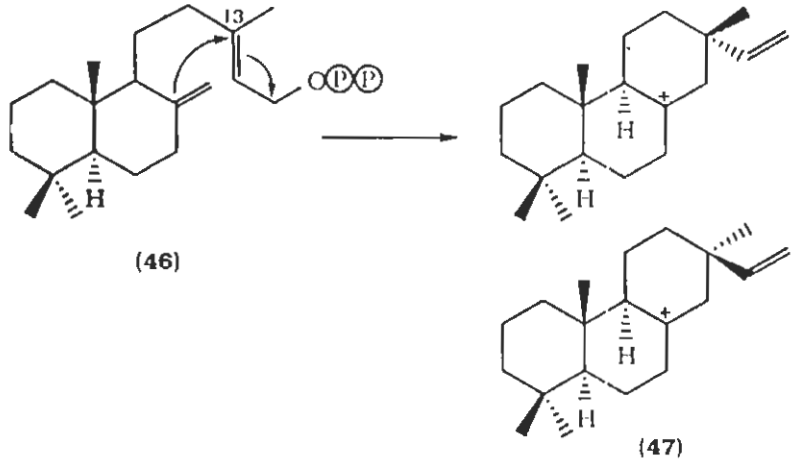
Among the bicyclic diterpenoids, labdanes (43) are most abundant group, and their biogenesis is the continuation of cyclization of the second ring of GGPP (Scheme-1.9). On the other hand, clerodanes (**44**), which exist in both antipodal types, are predominant in the forms which are derived from ent-labdane (**45**) (Scheme-1.9) (61).



Scheme-1.9: Biogenesis of bicyclic diterpenoids from GGPP.

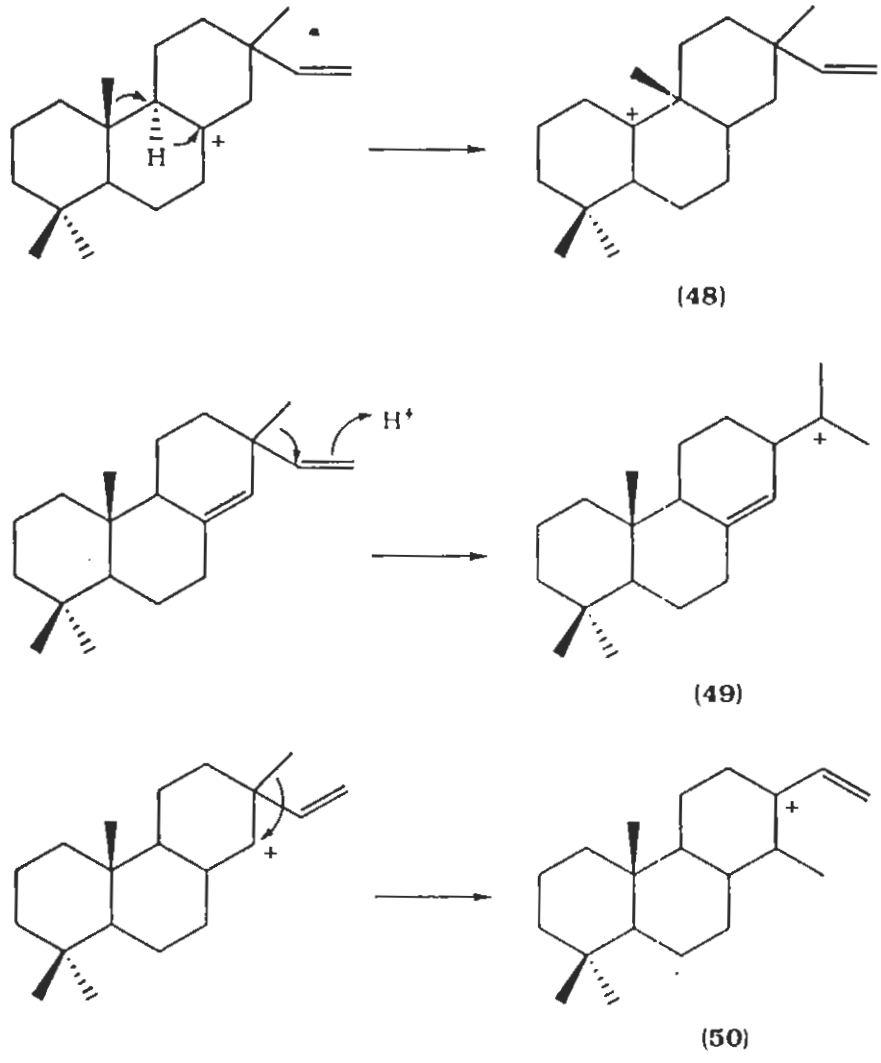
1.6.4 Tricyclic Diterpenoids

Copalyl pyrophosphate (**46**), obtained from labdane (**43**), is a precursor for the tricyclic diterpenoids, pimarane (**47**) via the internal attack of the double bond to C-13 which produces both of the epimers of pimarane (50, 55).



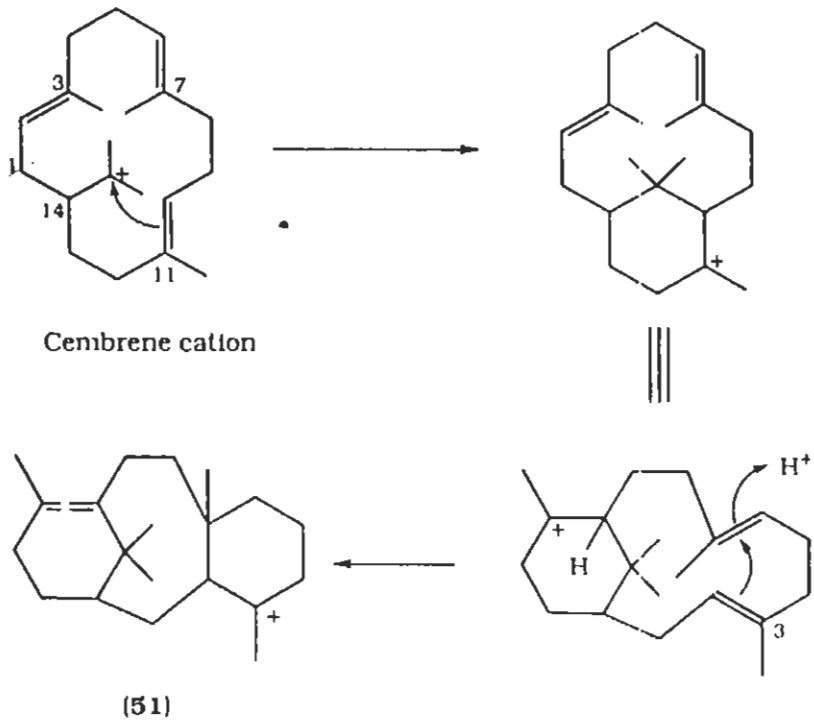
Scheme-1.10: Biogenesis of tricyclic diterpenoids.

Rosane (**48**), abietane (**49**) and cassane (**50**) can be made from a series of hydride and methyl shifts in pimarane skeleton, so they are considered as rearranged pimarane-type diterpenes (Scheme-1.11) (50).



Scheme-1.11: Rearranged pimarane type diterpenoids.

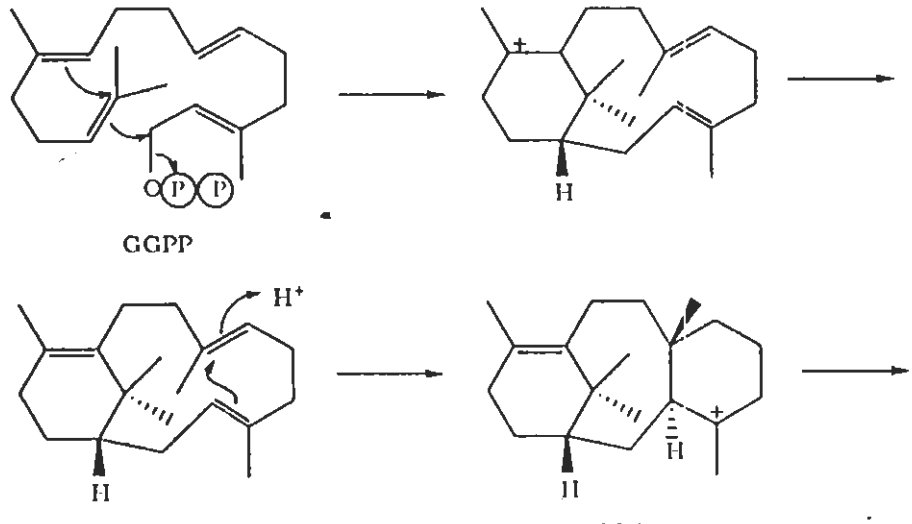
The taxenyl cation (**51**) precursor of taxol (**52**), the most important anti-cancer diterpenoid, can be biosynthesized through a cembrane pathway (Scheme-1.12) (50) or directly from GGPP (Scheme-1.13) (55).



Cembrene cation

(51)

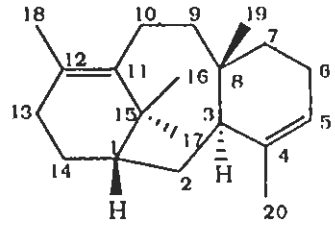
Scheme-1.12: Biogenises of taxol from cembrene.



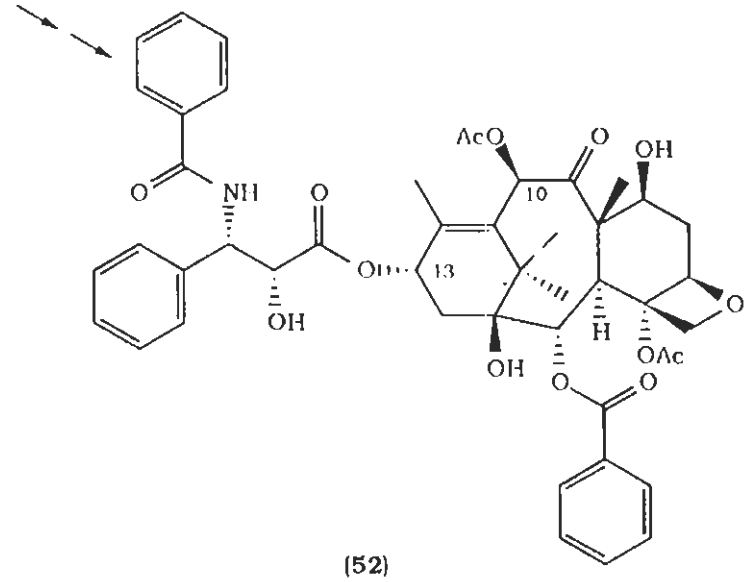
GGPP

verticillene

(51)



taxa-4(5), 11(12)-diene

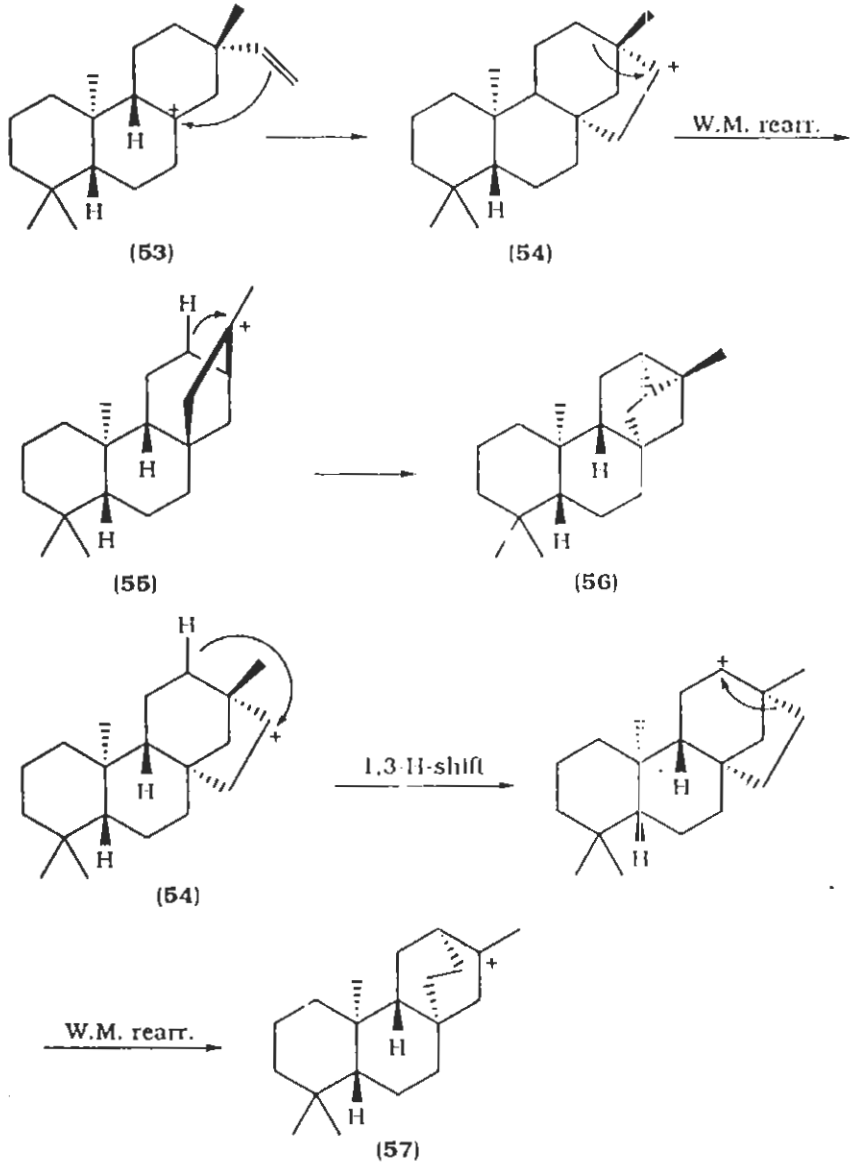


(52)

Scheme-1.13: Biogenesis of taxol from GGPP.

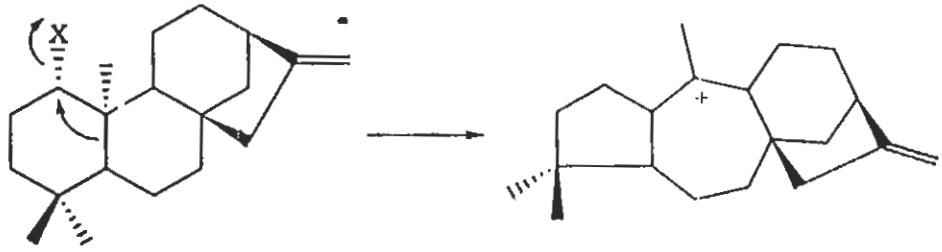
1.6.5 Tetra- and Pentacyclic Diterpenoids

Further cyclization of the tricyclic pimarenyl cation (**53**) generates the beyerane cation (**54**)- the precursor of several tetra- and pentacyclic diterpenes. A Wagner-Meerwein rearrangement in the beyerane cation produces the kaurane skeleton (**55**) which can be cyclized to trachylobane (**56**). Another important diterpene skeleton, atisane (**57**), can be produced from the beyerane skeleton (**54**) via a 1,3 H-shift and Wagner-Meerwein rearrangement (Scheme-1.14) (50,62).



Scheme-1.14: Biogenesis of tetra- and pentacyclic diterpenoids.

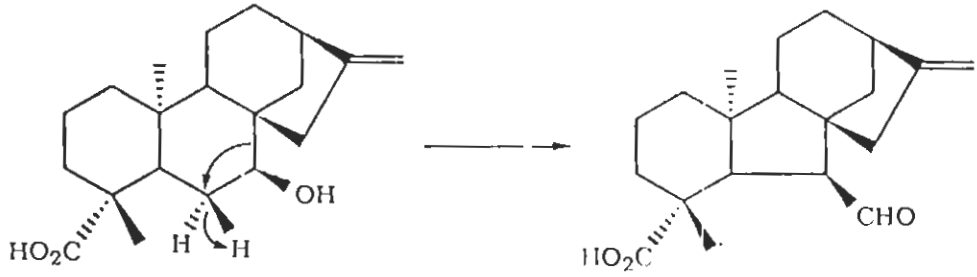
Andromedane-type diterpenes (**58**) are kaurane derivatives which are produced via expansion of the B ring of the kaurane skeleton (Scheme-1.15) (50, 55).



(**55**) (**58**)

Scheme-1.15: Rearrangement of the kaurane to the andromedane skeleton.

The biosynthesis of the gibberellins (**59**), the most important class of diterpenes which are found in almost all green plants, involves ent-kaurane (**60**) as its precursor via the construction of ring B of a kaurane intermediate (Scheme-1.16) (50, 55).



(**60**) (**59**)

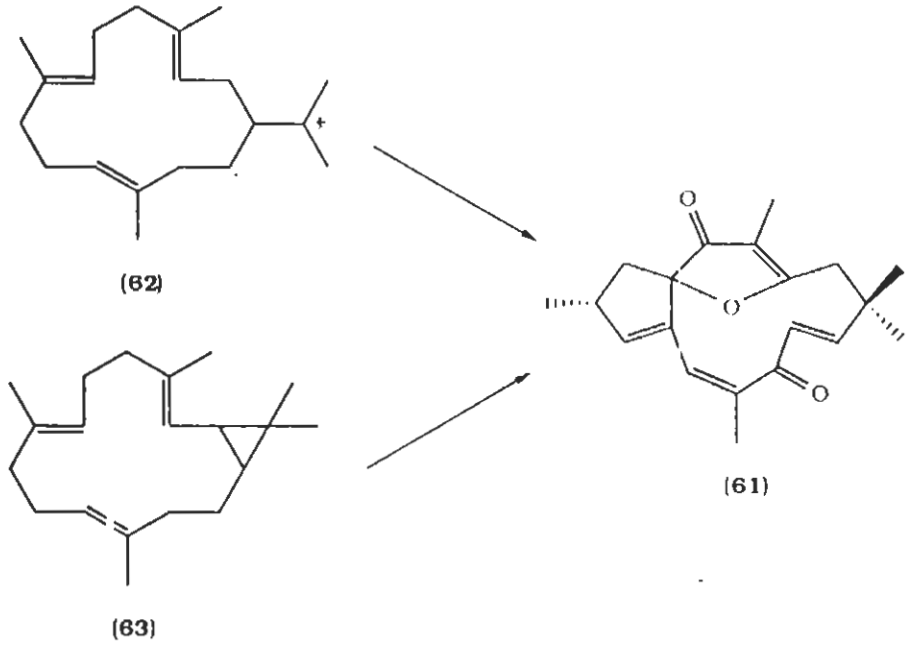
Scheme-1.16: Formation of the gibberellane from the kaurane skeleton

1.6.6 Poly- and Macrocyclic Diterpenoids

Investigation showed several different diterpenes with interesting biological activities in the plants of families Euphorbiaceae and Thymelaeaceae with tigliane, daphnane, ingenane, lathyrane,

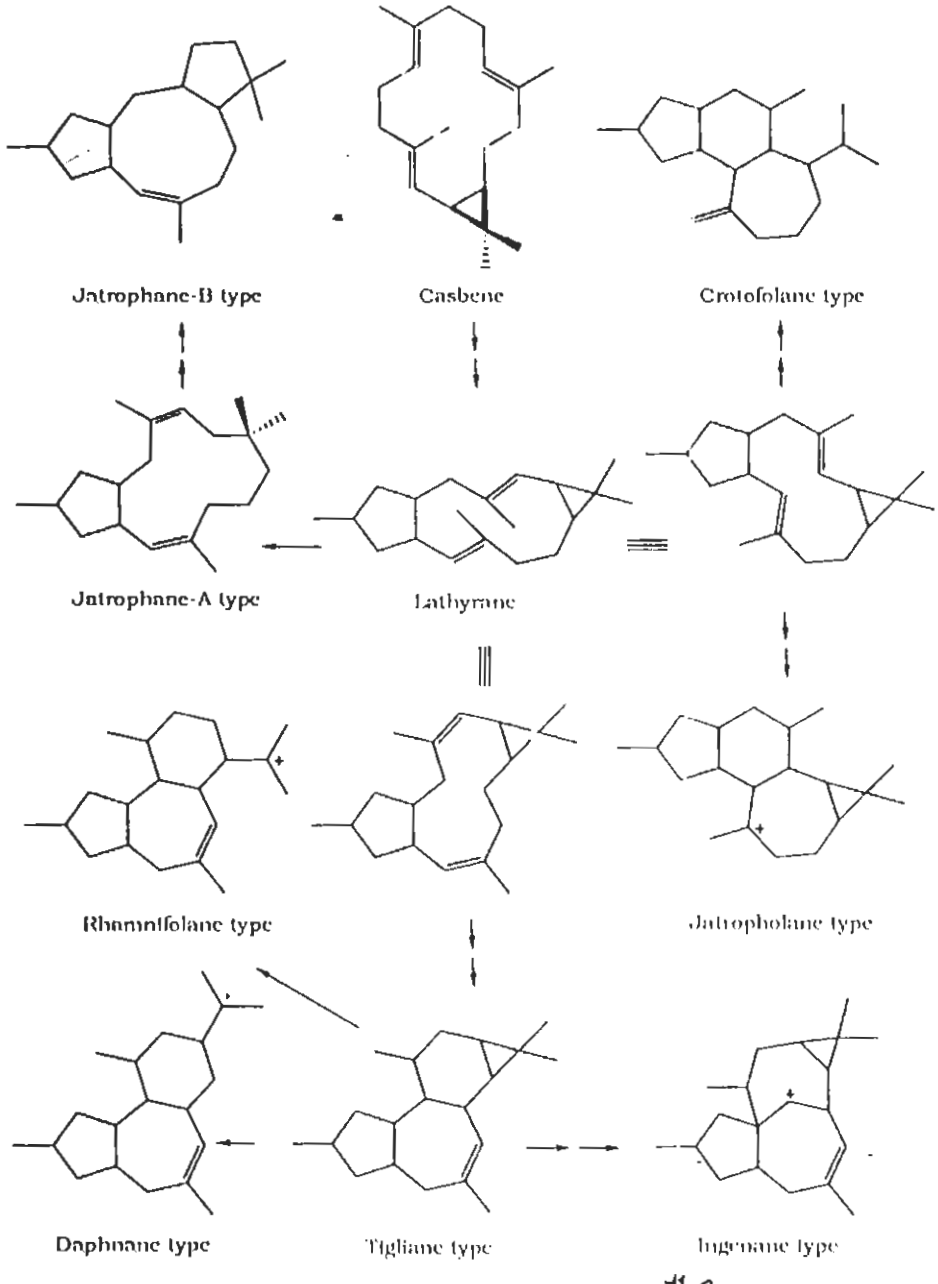
jatrophane, casbane, crotofolane and jatropholane skeleta. Besides these common skeleta, recently some other novel skeleta of lathyrane based polycyclic diterpenes were reported in literature which will be described in this section.

The macrocyclic diterpene, casbene, is considered as a precursor for the polycyclic diterpenes. According to Adolf and Hecker, jatrophane skeleton (**61**) can be produced from cembrene cation (**62**) as well as from casbene (**63**) (Scheme-1.17) (63).



Scheme-1.17: Biogenesis of jatrophane.

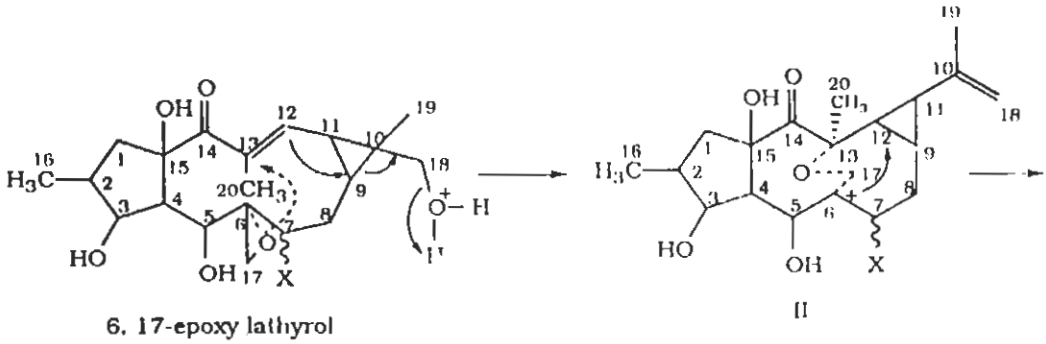
Lathyrane can be considered as a cyclization product of casbene. The tigliane, ingenane and daphnane skeleta may be obtained by further cyclization and rearrangment of the lathyrane skeleton (Scheme-1.18) (50, 55, 63).



Scheme-1.18: Biogenetic relationship between diterpenoids of Euphorbiaceae and Thymelaeaceae (50).

Myrsinol ester with five, seven and six-membered rings was isolated from *Euphorbia myrsinites* (54) and proposed to be biosynthesized from a 6,17-epoxy lathyrol derivative (**64**) (64). The protonation of the C-18 hydroxyl group, opening of the C-9/C-10 bond of the three-membered ring, and

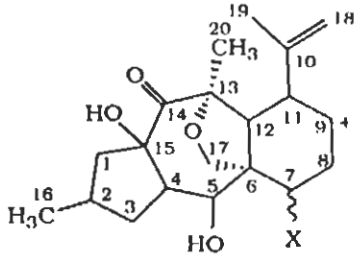
attack of lone pair electrons on oxygen of 6,17-epoxide to C-12/C-13 double bond produces the intermediate II. This can be followed by attack of well-oriented C-9/C-12 bond of cyclopropane ring to the carbonium ion at C-6 in II. Finally, dehydrogenation between C-9 carbonium and C-8 produces myrsinol skeleton (**65**), (Scheme-1.19).



6, 17-epoxy lathyrol

(**64**)

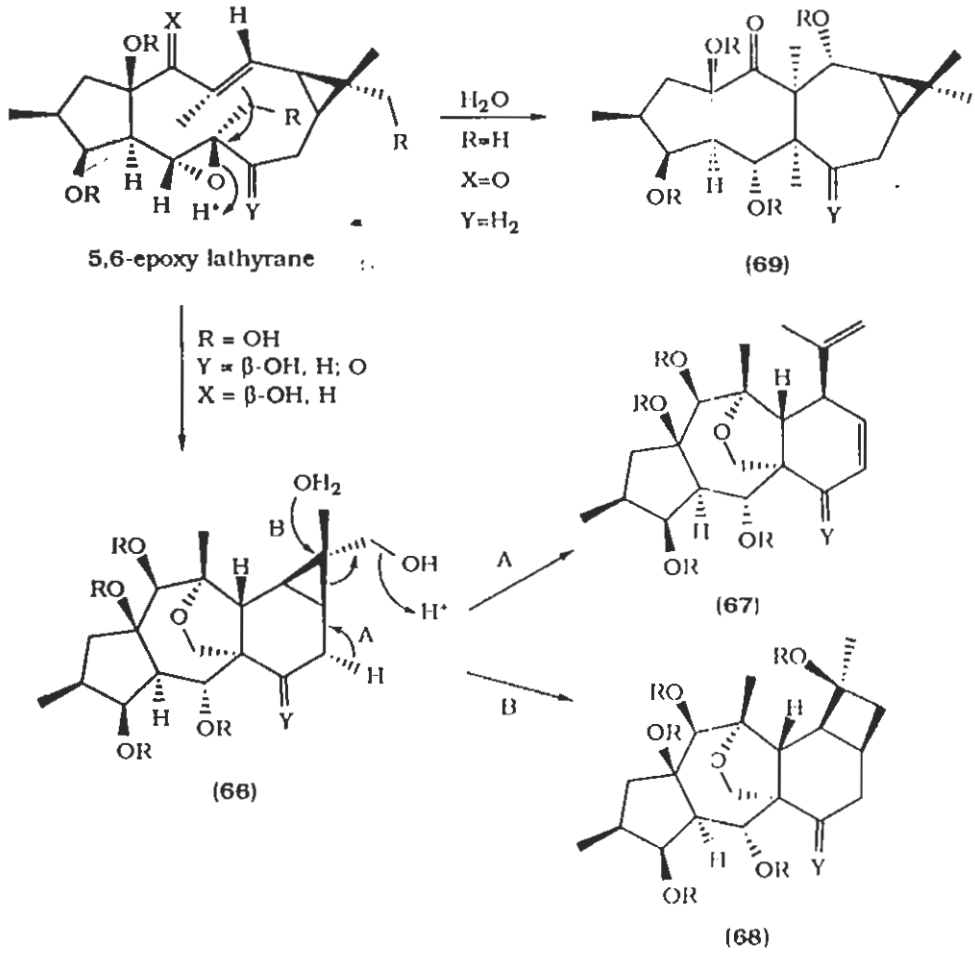
II



(**65**)

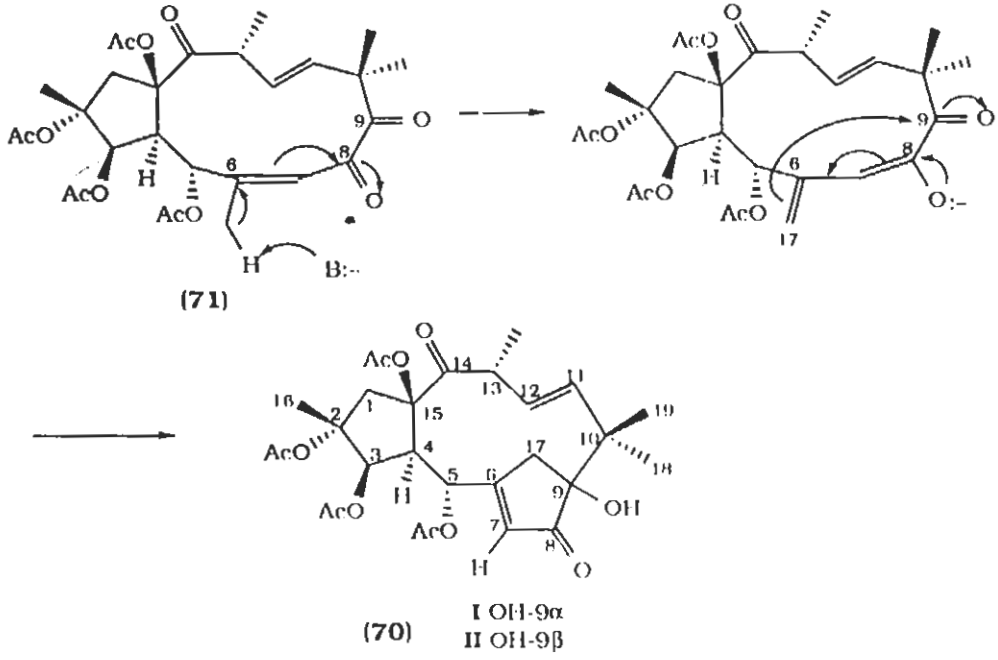
Scheme-1.19: biogenesis of myrsinol ester.

In another approach, the opening of epoxide ring by attack of Δ^{12} double bond, followed by closure of oxyrane ring in **66** and then opening or expansion of the three membered ring can produce myrsinol skeleton (**67**) and cyclomyrsinol ester (**68**). Euphoractin B (**69**) can be synthesized in the first step without simultaneous attack of C-5 hydroxy group to C-13 (65) (Scheme-1.20).



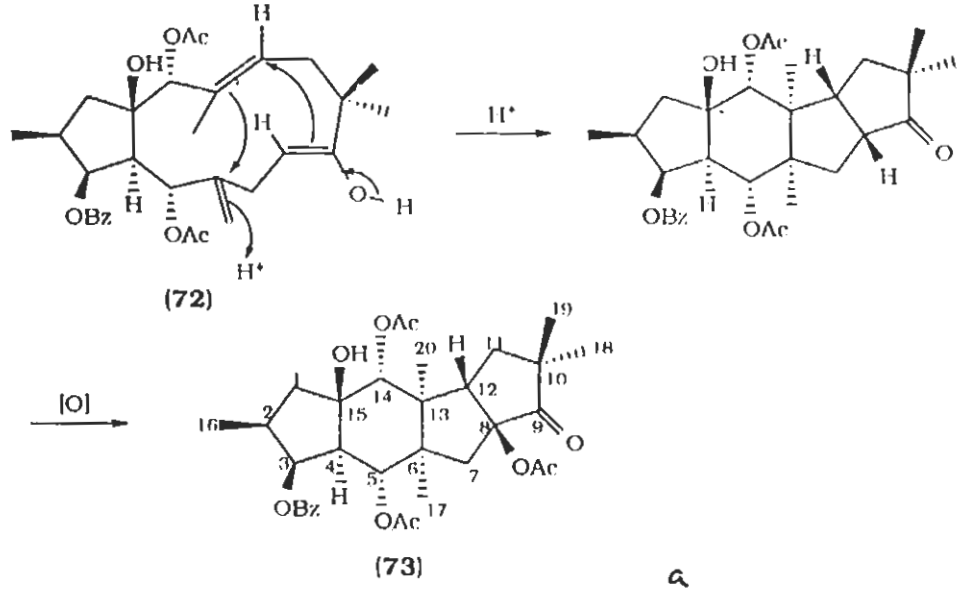
Scheme-1.20: Biogenesis of euphoractin and myrsinol.

Euphoperfoliane (**70**), an 11-membered macrocyclic ring diterpenoid, was isolated from *Euphorbia semiperfoliata* (66). The biogenetic pathway for the formation of this compound might be through an intramolecular vinylogous aldol condensation reaction of the Δ^6, C-8, C-9-dione-type jatrophane (**71**) nucleus (Scheme-1.21) (66).



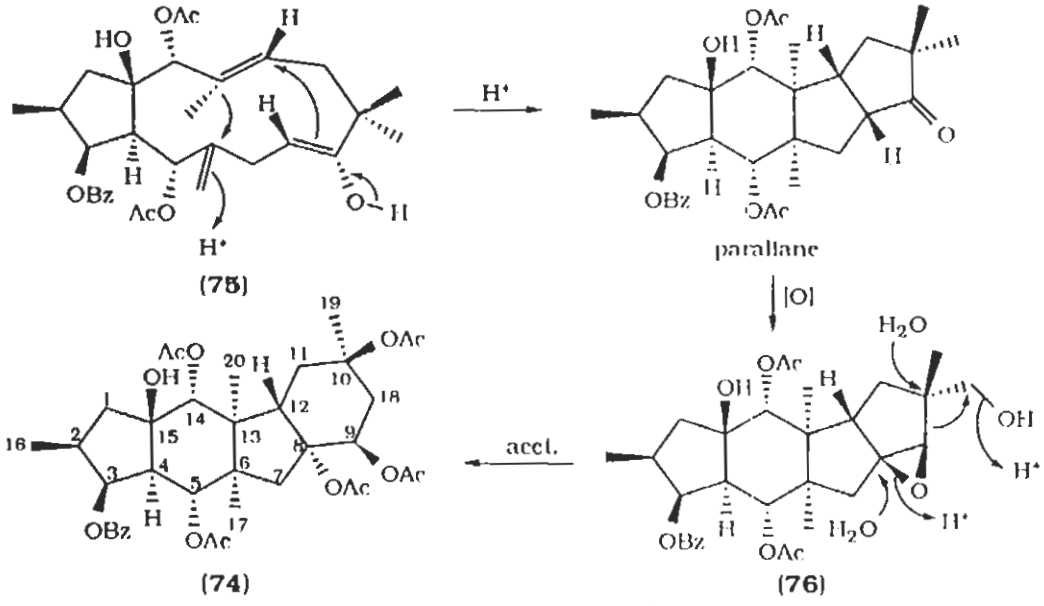
Scheme-1.21: Biogenesis of euphoperfoliane.

Another polycyclic diterpenoid with a polyquinane type skeleton which was suggested to be synthesized from a jatrophane skeleton (**72**), is paraliane (**73**), this diterpenoid was separated from *Euphorbia paralias* (67). The tetracyclic skeleton of paraliane is produced in one step (Scheme-1.22).



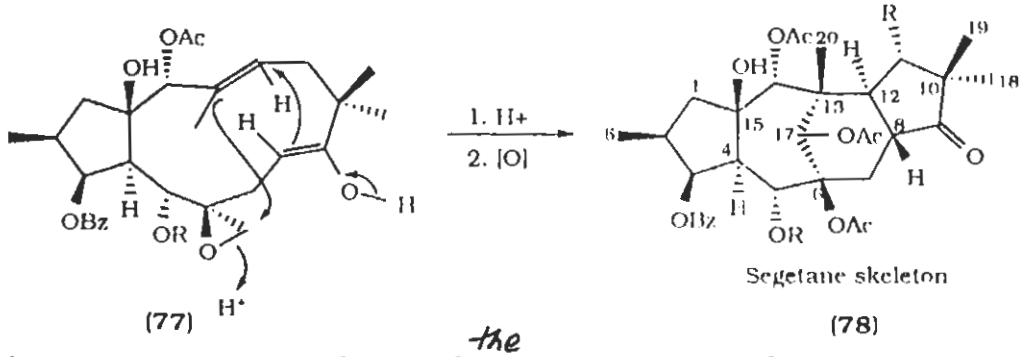
Scheme-1.22: Biogenesis of paraliane from jatrophane precursor.

The tetracyclic diterpene skeleton pepluane (**74**) was isolated from *Euphorbia peplus*. It can be derived from a jatropha-6(17), 12-diene (**75**), the basic skeleton of paraliane. The oxidation of a methyl group at C-10 could give the precursor (**76**) for expansion of a five membered to six-membered D ring, and the 8α, 9β-diol could be formed via a 8β, 9β-epoxide (68) (Scheme-1.23).



Scheme-1.23: Biogenetic pathway for the synthesis of pepluane.

The segetane diterpenoids were isolated from *Euphorbia segetalis* (69). Cyclization of a jatrophane precursor (**77**) followed by oxidation at C-17 is suggested as the biogenesis for the segetane diterpenoids (**78**) (69) (Scheme-1.24).



Scheme-1.24: Biosynthesis of the segetane skeleton from jatrophane in one step.

1.7 A Brief Introduction on the Essential Oils

1.7.1 General Introduction

Essential oils, ethereal oils, or volatile oils are the volatile constituents of a plant, which can be extracted by steam distillation. They are oily material, insoluble in water, soluble in alcohol and ether, and are distinguished from fixed or non-volatile oils by their volatility. A spot of an essential oil on a piece of paper will evaporated after some time, unlike a fixed oil, which remains for a long period of time (70, 71).

About 200 essential oils are known commercially, among which twenty five are produced on a large scale, for instance citrus, peppermint, spearmint, orange, lemon and some spice oils, such as clove and nutmeg. The essential oils are widely used in different industries, for example as ingredients in many pharmaceutical products, from antiseptics and flavouring agents to analgesic and antimicrobial components in mouth washes or gargles. In fragrances, perfumes and different foodstuffs the essential oils are important ingredients (72, 73).

1.7.2 The Sources of the Essential Oils

Essential oils can be prepared from almost every part of a plant, for instance, some of them are biosynthesized in flowers (rosemary and jasmine), in fruits (fennel, orange, lemon, star anise), in flower buds (clove oil), in seeds (mustard), in leaves (geranium, mint, sage), in woods

and inner bark of the shoots (camphor, cinnamon), and they can be extracted from gum (turpentine oil) (72, 74).

1.7.3 Methods for the Extraction of the Essential Oils

1.7.3.1 Steam and Hydrodistillation

The essential oils can be extracted by different methods, among them, steam and hydrodistillation of the plant material are the most important methods. In steam distillation, a mixture of the volatile substances and steam, which is produced in a separate boiler, are condensed in a condenser and separated subsequently.

In the hydrodistillation method a mixture of the plant material and water are heated. In the case of substances which are sensitive to water it is not a good method, but for dry plant material, it has great application. Figures 1.4 and 1.5 show two different glassware systems for the extraction of the oils by the hydrodistillation method, first the British pharmacopoeia, and second, the Clevenger apparatus (75).

1.7.3.2 Extraction with Solvent

Extraction with a solvent is used for very sensitive plant materials such as flowers. The plant material is extracted by soaking in a non-polar solvent like hexane at room temperature, or they can be extracted by Soxhlet several times. After removing the solvent at reduced pressure the viscous residue is named concrete. The concrete contains both volatile and non-volatile/non-polar compounds and, after

dissolving in ethanol, the non-polar/non-volatile materials are precipitated which can be separated by filtration. The filtrate, after concentration under reduced pressure, is named absolute and it contains essential oil. This method has applications in comparison to steam distillation (70).

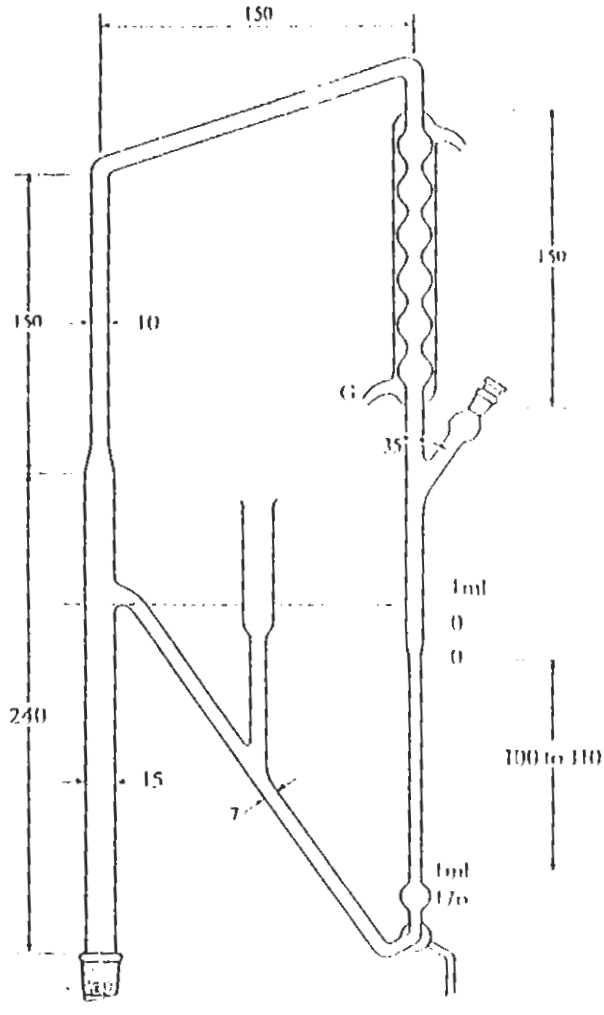


Figure 1. 4: British Pharmacopoeia apparatus for the hydrodistillation of essential oils

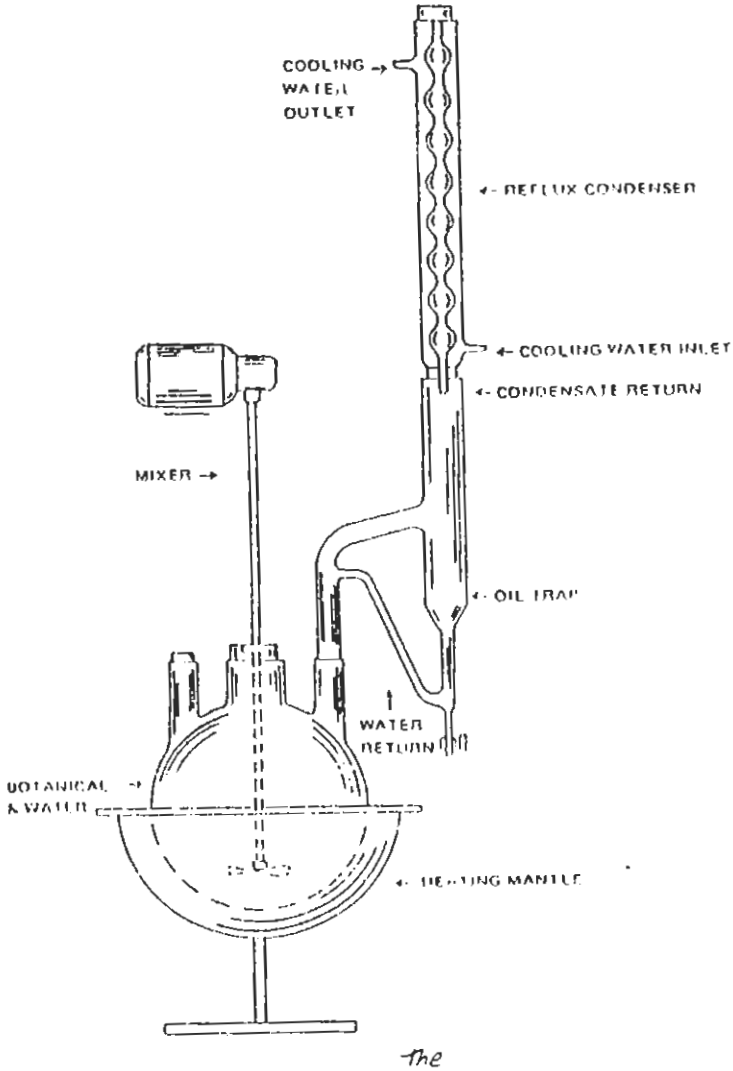


Figure 1. 5: Clevenger apparatus for extraction of essential oils (Courtesy Fritzsche Dodge & Olcott, Inc.)

1.7.3.3 Extraction of the Essential Oil Using Supercritical Fluid Extraction (SFE)

In 1879 Hanny and Hogarth demonstrated the solubilizing properties of supercritical fluids. Recently, this method of extraction has been applied for different classes of natural products, including essential oils.

The advantages of carbon dioxide (CO_2) supercritical fluid extraction for isolation of the essential oils over steam-and hydrodistillation and solvent extraction are the mild condition, low price, lack of toxicity and the absence of problems for the disposal of the solvent (76).

Figure 1.6 shows the schematic diagram of a home made SFE apparatus in which the plant material can be extracted by fluid CO_2 at different densities, controlled by pressure and temperature of the CO_2, this permits to determination of the nature of the extract (77).

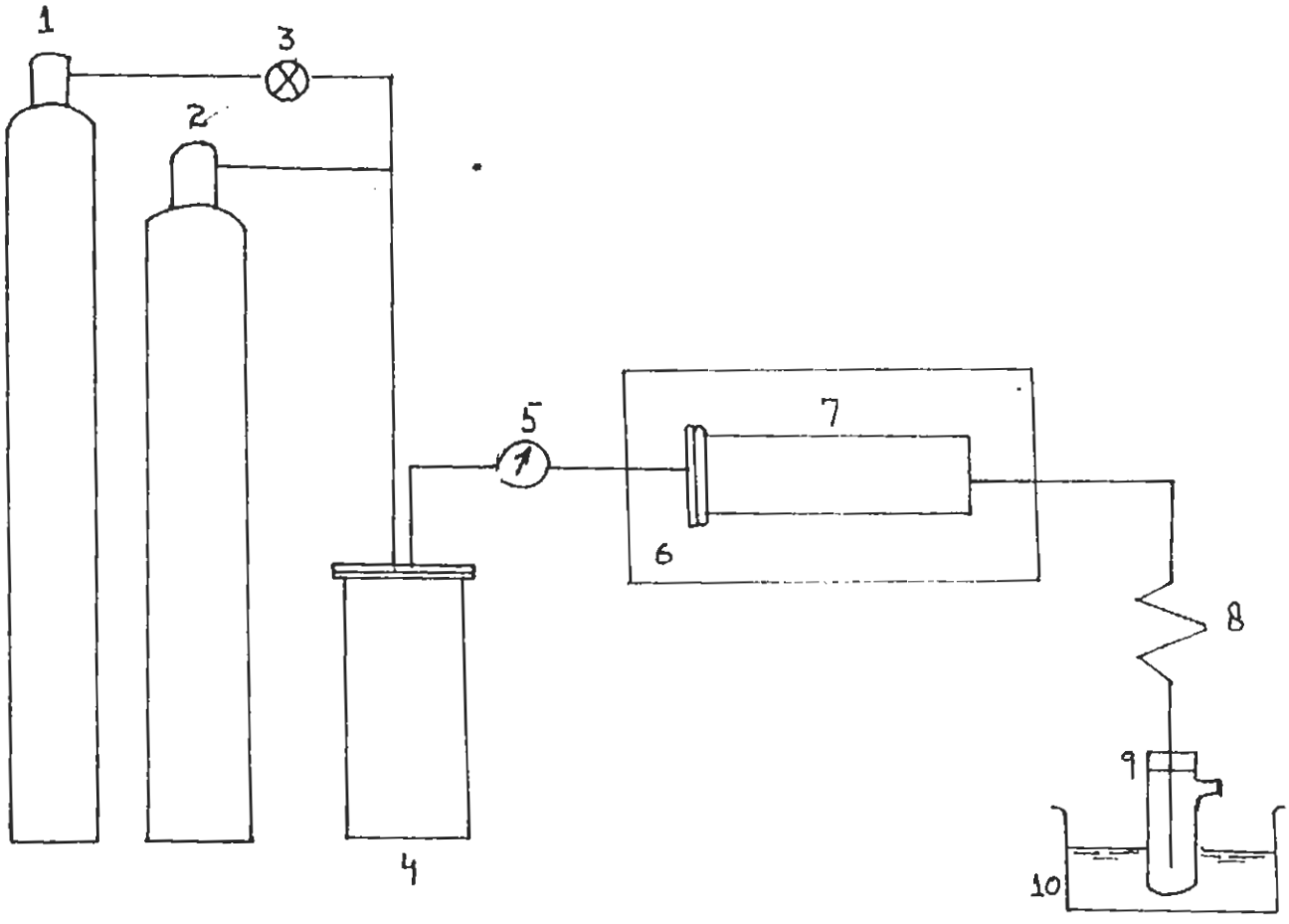


Figure 1. 6: Schematic diagram of a home made SFE system:
1. N_2 tank; 2. CO_2 tank; 3. valve; 4. high pressure vessel;
5. pressure meter; 6. oven; 7. extraction vessel; 8. restrictor;
9. collection vessel; 10. water bath.

1.7.4 Chemical Composition of the Essential Oils

The distinction between essential oils and flavour and fragrant compounds is not at all clear, but the essential oils are typically volatile terpenes and esters, while the flavour and fragrance compounds mostly consist of other volatiles like phenols, alkanes, aldehydes, etc. The sulfur and nitrogen-containing compounds which are available to be extracted by steam distillation also can be considered as components of essential oils (73, 76).

1.7.5 Qualitative and Quantitative Analysis of the Essential Oils

The most important method for the identification of the essential oils, which may contain several constituents, is gas chromatography (GC) and its combination with mass spectrometry (GC/MS), in which even a trace amount of a compound can be identified. This method is applicable for the identification of those compounds which have been identified previously, and for which GC and mass spectral data are available for comparison (78).

For the determination of the structure of a new compound, it must be first purified and then utilizing a variety of different spectroscopic techniques such as NMR, MS, etc., be elucidated. For this purpose, several methods including vacuum distillation, flash chromatography, and preparative gas chromatography must be conducted in order to obtain a pure compound (79).

The other methods including the coupling of GC and Infrared spectroscopy (GC/IR), and Co-injection with authentic samples in gas chromatography are also applied (80).

2. RESULTS AND DISCUSSION

2.1 Part A: Phytochemical Investigation of *Euphorbia decipiens* Boiss. & Buhse

2.1.1 Introduction

Euphorbia decipiens Boiss. & Bushe, belonging to the family Euphorbiaceae, grows wild in different parts of Iran at high altitudes and is indigenous to Iran (5). According to the best of our knowledge, the chemical constituents of this plant have not been investigated so far.

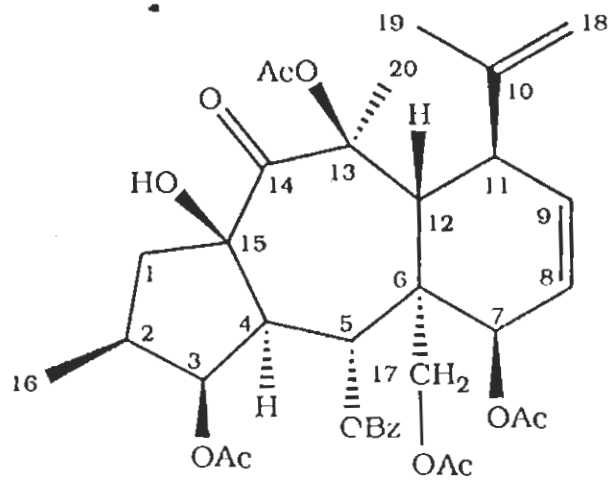
The medicinal properties attributed to the genus *Euphorbia* (see the Introduction section) prompted us to investigate *E. decipiens* for its chemical constituents.

Investigation of the chloroform-methanol (1:1) extract of the whole plant, resulted in five new diterpene esters with a novel skeleton, related to the lathyrane or myrsinol type skeleton and named as: decipinone (**79**), isodecipinone (**84**), decipidone (**85**), isodecipidone (**86**) and decipinone A (**87**). Two novel tetracyclic diterpenoids: decipinol ester A (**88**) and B (**90**), two new pentacyclic diterpene esters, karajinon A (**91**) and B (**94**) were also isolated and found to have a lathyrane related skeleton.

The known compounds isolated from this plant for the first time were: β-sitosterol (**95**), cycloeucalenol (**96**), obtusifoliol (**97**), cycloart-23-en-3,25-diol (**98**), and 24-methylenecycloartan-3β-ol (**99**).

2.1.2 Decipinone (79)

Decipinone (79) was obtained as colorless crystals . . . . grown from methanol by slow evaporation of the solvent at room temperature.



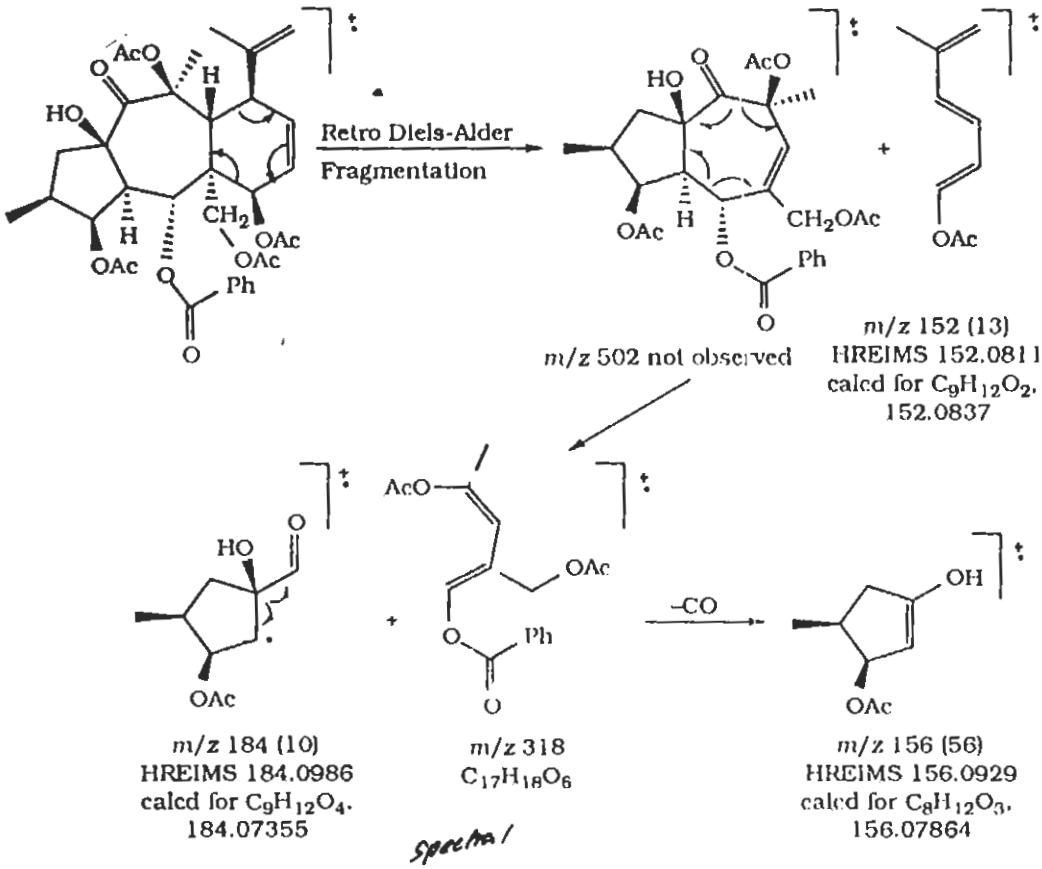
(79)

2.1.2.1 Structure Elucidation of decipinone (79)

Decipinone (79) was assigned the molecular formula $C_{35}H_{42}O_{12}$ on the basis of HREIMS (obsd. 654.2698, calcd. 654.2676). In the EIMS spectrum the ions at *m/z* 594, 534, 474 and 414 indicated the presence of acetate groups which were eliminated from the molecular ion at *m/z* 654 in the form of acetic acid. The base peak at *m/z* 105 $[C_6H_5CO]^+$, and others at 121 $[C_6H_5CO_2]^+$ and 533 $[M-121]^+$ indicated the presence of a benzoate ester group in the molecule (81).

The fragmentation pattern of the mass spectum of decipinone is illustrated in Scheme-2.1. Retro-Diels Alder fragmentation in the six membered ring gives the ions at *m/z* 152 and 502. The ions at *m/z* 318 and 184 can be obtained from the ion at *m/z* 502 and the ion at *m/z* 156 may be obtained by the loss of CO from the ion at *m/z* 184. The fragmentation pattern was confirmed by the HREIMS spectrum. The

at *m/z* 502 and 318 were not observed, possibly because of the low stability of the ions, caused by the presence of several ester groups.



Scheme-2.1: The mass fragmentation pattern for decipinone.

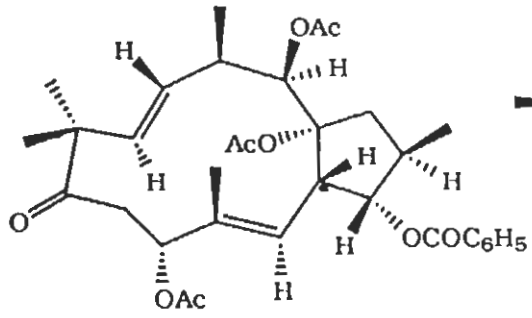
The IR spectrum showed characteristic peaks for carbonyl groups at 1700-1740 cm^{-1}. The absorptions at 1580, 1600 and 710 cm^{-1} represent the phenyl and and a sharp peak at 3500 cm^{-1} indicated a non-hydrogen-bonded hydroxyl group in the molecule (81).

The ^{1}H-NMR spectrum of **79** (Table-2.1) showed four singlets for the acetate methyl groups at δ 2.12, 2.07, 1.94 and 1.73. The high field shift of the last peak may be due to anisotropic effect which has been

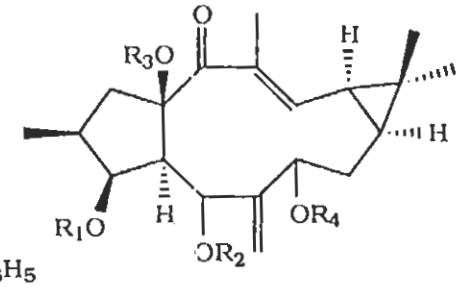
observed earlier also by other authors e.g. in euphoscopin B (**80**), and 7-hydroxy-lathyrol (**81**) (82, 83). There were three signals due to protons geminal to ester groups which were observed as a doublet at δ 6.38 (d, *J*=11.5 Hz, H-5), a multiplet at δ 4.87 (H-7, overlapped with 2 x H-18 signal) and a triplet at δ 5.3 (t, *J*=3.5 Hz, H-3).

The spectrum also showed three methyl signals in the molecule comprised of a secondary methyl at δ 0.89 (d, *J*=6.7 Hz, 3 x H-16), an olefinic methyl at δ 1.77 (s, 3 x H-19) and a tertiary methyl at δ 1.67 (s, 3 x H-20) which appears to be geminal to an oxygen bearing group. This last chemical shift is quite near to that observed for the corresponding methyl (Me-20), in the ^{1}H-NMR spectrum of myrsinol ester (**82**) at δ 1.58 (4).

The vicinal olefinic protons showing signals at δ 5.72 (dd, *J*=4.5, 9.5 Hz, H-9) and at δ 5.96 (ddd, *J*=2.0, 6.5, 9.5 Hz, H-8) are separated by a methine proton at δ 3.28 (br ddd, *J*=2.0, 4.5, 8.0 Hz, H-11) from the terminal olefinic protons at δ 4.87 in an isopropenyl group, H-18. The downfield chemical shift of H-11 can be considered as a consequence of its location between the two double bonds (4). This proton (H-11) was also coupled with an unusually downfield proton at δ 4.07 (*J*=8.0 Hz, H-12) which appears to be located in the anisotropic field of carbonyl esters or the lone pair of electron of the free hydroxyl group (82, 84).

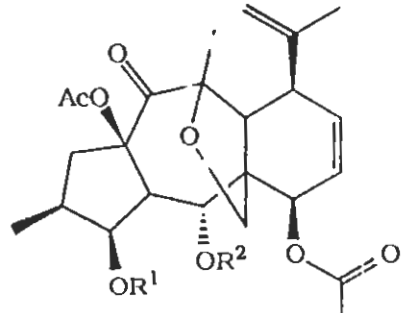


(80)



(81)

R_1–R_4=2 $COCH_3$, 2 COC_6H_5



a R^1=R^2=Pr
b R^1=Pr, R^2=Bu
c R^1=Bu, R^2=Pr
d R^1=R^2=Bu

(82)

Pr=Propionyl
Bu=Butanoyl

Two of doublets at δ 3.97 (d, *J*=12.0 Hz, H-17) and 4.34 (d, *J*=12.0 Hz, H-17') were also observed in the ^{1}H-NMR of **79** which represent an oxymethylene group in this molecule.

Besides the ^{1}H-^{1}H COSY spectrum (Fig. 2.1) for deducing the correlations between protons, the vicinal relationships, which were not very clear in ^{1}H-NMR because of overlapping of the different protons were examined by spin decoupling experiments. Irradiation of the multiplet at δ 2.1 (H-2) collapsed the signals of δ 0.89 (d, *J*=6.7 Hz, H-16), δ 3.15 (dd, *J*=9.5, 14.5 Hz, H-1α) and δ 5.3 (t, *J*=3.5 Hz, H-3) to a singlet and two doublets, respectively. On the other hand, irradiation at δ 4.87 changed the signals at δ 5.96 (ddd, *J*=2.0, 6.5, 9.5 Hz, H-8) to a doublet

of doublets indicating the overlapping of H-7, with the olefinic proton, 2 x H-18. Irradiation at δ 4.07 collapsed H-11 to a broad doublet indicating the vicinal coupling of H-11 and H-12.

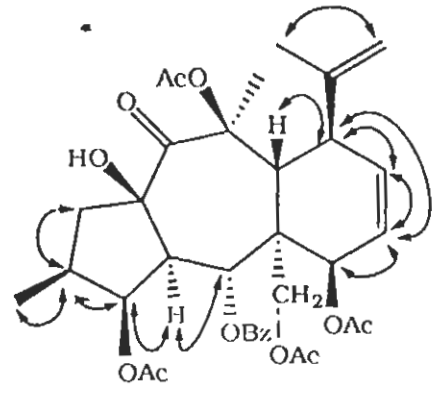


Figure-2.1: ^{1}H-^{1}H COSY 45° correlations for decipinone (**79**).

The ^{13}C-NMR spectra (BB and DEPT) of **79** (Table 2.1), showed 33 signals due to 35 carbons including seven CH_3, three CH_2, twelve CH and eleven quaternary carbons, of which eight were oxygen bearing (one tertiary alcohol, one tertiary ester, one ketone and five ester carbonyls).

The signals at δ 79.4 (d, C-3), 70.8 (d, C-5) and 67.9 (d, C-7) represented the ester bearing methine groups. The downfield signals at 122.2 (d, C-8), 136.0 (d, C-9), 113.1 (t, C-18) and 147.1 (s, C-10) suggested the presence of two double bonds in the molecule, of which one is terminal (C-10/C-18). Other characteristic peaks were detected at δ 86.5, 86.6 (s, C-13, C-15) and 205.6 (s, C-14) and a triplet at δ 62.3 which represented an oxymethylene bearing carbon atom. All the direct carbon-hydrogen connectivities were confirmed by observing the corresponding cross peaks in the HMQC spectrum (Table-2.1). The relative location of the acetate and benzoate groups were deduced by observing the cross peaks between the corresponding protons and carbonyl carbons of the ester

groups in the HMBC spectrum which was in the case of benzoyl carbonyl carbon at δ 165.1 with H-5, and for other acetate groups at about δ 170 with H-3, H-7 and H-17 (Table-2.1) (85). The cross peaks between Hα-1 and C-14 and C-15, for H-12 with C-6, C-10, C-13 and C-17, and for H-17 with C-5, C-6 and C-12 , were especially helpful to assign the structure of decipinone.

With the aid of the above mentioned NMR experiments, including COSY, HMQC and HMBC, two partial structures A and B were suggested for this molecule which were connected through fragment C to produce structure E (Fig. 2.2).

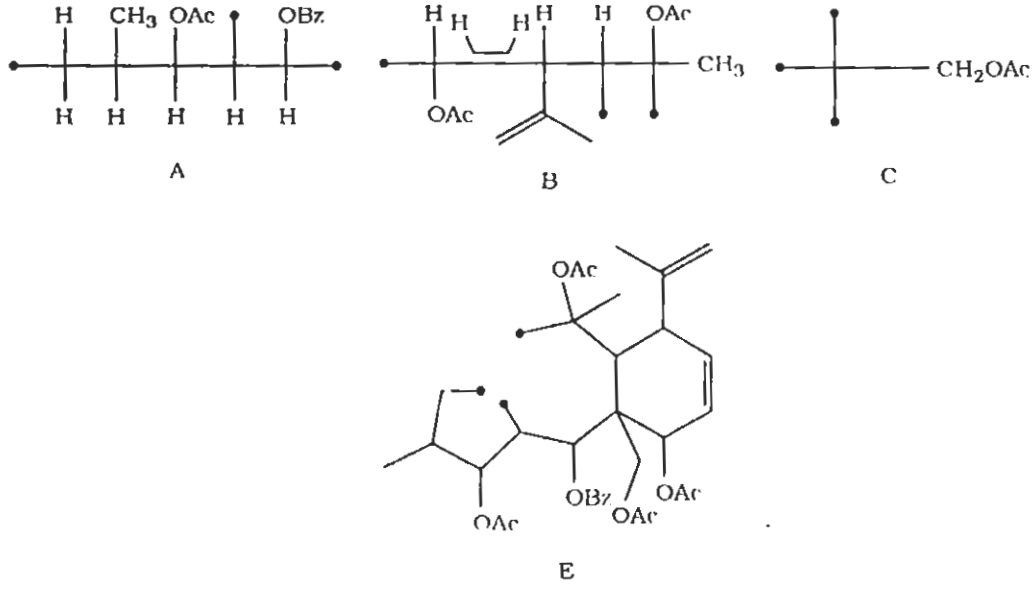


Figure-2.2: Partial structures suggested by different NMR spectroscopic techniques for decipinone (**79**).

According to the molecular formula $C_{35}H_{42}O_{12}$, **79** contains fifteen degrees of unsaturation. Out of these, twelve are accounted for by the double bond(two), acetyl groups (four), benzoyl group (five) and a ketone (one) group. Therefore **79** contains three rings, one of these is six membered as suggested above (Fig. 2.2). The very close chemical shifts of C-13 (δ 86.5) and C-15 (δ 86.6) indicate that they are part of a seven-

membered ring separated by a carbonyl group at C-14. The H-1α signal (δ 3.15) is shifted downfield due to the anisotropic effect of the carbonyl group at C-14. Such downfield shifts have been observed in other similar compounds e.g. 7-hydroxy-lathyrol (**81**) (83), myrsinol esters (**82**) (4, 54) and esulatins A-C (20). The chemical shifts of the protons in the ^{1}H-NMR spectrum of 7-hydroxy-lathyrol (**81**) (83) at positions H-3, H-4 and Me-16 were in a good agreement with the corresponding signals in the ^{1}H-NMR spectra of **79** (Table-2.1). Also, in the ^{1}H and ^{13}C-NMR data recorded for the esters of the myrsinols (**82**) (4, 54) the diterpenes isolated from *E. myrsinites* were found to be similar to those recorded for **79**, therefore, the carbon skeleton of **79** was considered as a tricyclic lathyrane skeleton. Both HMBC and HOHAHA (Fig. 2.3) experiments confirmed this structure. In the HOHAHA spectra the presence of the three spin systems (A, B and C) were further confirmed. (Fig. 2.3).

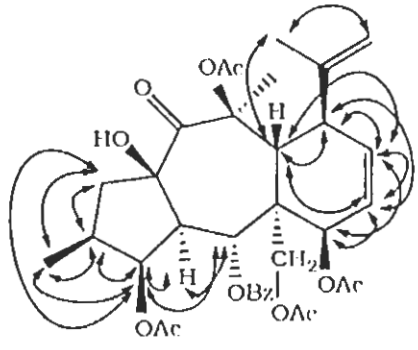


Figure 2.3: Long-range ^{1}H-^{1}H correlations HOHAHA for **79**.

Table-2.1 Spectral data of decipinone (79)*.

Position	^1H	^{13}C	HMBC Correlations
1α	3.15 dd (14.5, 9.5)	45.2 (t)	C-2, C-3, C-4, C-14, C-15
1β	1.60 dd (14.5, 11.5)		
2	2.10 m	37.7 (d)	
3	5.30 t (3.5)	79.4 (d)	$OCOCH_3$, C-1, C-15
4	2.36 dd (11.5,3.5)	53.6 (d)	C-5, C-6, C-14
5	6.38 d (11.5)	70.8 (d)	OCOPh, C-4, C-6, C-7, C-17
6	-	43.3 (s)	
7	4.87 m	67.9 d)	$OCOCH_3$, C-5, C-6, C-8, C-9
8	5.96 ddd (2.0, 6.5, 9.5)	122.2 (d)	C-6, C-7, C-9, C-11
9	5.72 dd (4.5, 9.5)	136.0 (d)	C-7, C-11, C-12
10	-	147.1 (s)	
11	3.28 ddd (2.0, 4.5, 8.0)	45.9 (d)	C-6, C-8, C-9, C-10, C-12, C-13, C-18, C-19
12	4.07 d (8.0)	41.3 (d)	C-5, C-6, C-10, C-11, C-13, C-17
13[a]	-	86.5 (s)	
14	-	205.6 (s)	
15[a]	-	86.6 (s)	
16	0.89 d (6.7)	14.3 (q)	C-1, C-2, C-3
17	3.97 d (12.0)	62.3 (t)	$OCOCH_3$, C-5, C-6, C-7, C-12
17'	4.34 d (12.0)		$OCOCH_3$, C-5, C-6, C-7
18	4.87 m (2H)	113.1 (t)	C-9, C-10, C-11, C-19
19	1.77 s	20.0 (q)	C-10, C-11, C-18
20	1.67 s	23.3 (q)	C-12, C-13, C-14
$OCOCH_3$[b]			
	1.73 s	20.7 (q)	
	1.94 s	20.8 (q)	
	2.07 s	20.9 (q)	
	2.12 s	21.0 (q)	
$OCOCH_3$[b]			
	-	169.8 (s)	
	-	169.9 (s)	
	-	170.3 (s)	
	-	170.7 (s)	
Benzoyl			
1'	-	129.9 (s)	
2',6'	7.87 dd (1.5, 10.0)	129.7 (d)	
3',5'	7.37 br t (8.0)	128.3 (d)	
4'	7.50 tl (1.0, 8.5)	133.1 (d)	
7'	-	165.1 (s)	
OH	4.12 s	-	

*The ^1H-^{13}C connectivities and ^{13}C multiplicities were deduced according to HMQC and DEPT experiments.

[a,b]The assignments may be interchanged.

2.1.2.2 Determination of the Stereochemistry of decipinone (79)

To determine the relative stereochemistry at positions 3,4, 11 and 12, the coupling constants were examined. In the ^{1}H-NMR spectrum, the J value 8.0 Hz for H-12 indicated the trans relationship between H-11 and H-12. The doublet at δ 6.38 with a J value of 11.5 Hz for H-5 and a doublet of doublets at δ 2.36 (J=3.5, 11.5 Hz, H-4) showed that these two protons are again in an *anti* orientation to each other and a triplet at δ 5.3 (J=3.5 Hz, H-3) confirmed that this proton must be in a similar position with the same dihedral angle to H-2 and H-4, which is consistant with the stereochemistry shown for **79**.

In the NOESY spectrum (Fig. 2.4) cross peaks between δ 4.87 (H-7, H-18) with δ 4.07 (H-12) and δ 3.28 (H-11) were detected, through which we concluded that H-7 and H-11 must be on one face of the molecule, and H-12 and H-18 on the other. On the other hand, NOE difference spectroscopy, irradiation of Me-20 afforded NOEs at H-1α (4.6%), H-11 (7.3%), H-17 (2%) and H-17' (4.1%). Also, irradiation of H-12 and the hydroxy proton at C-15 provided a significant enhancement at H-5 which led us to consider the stereostructure **79** for decipinone.

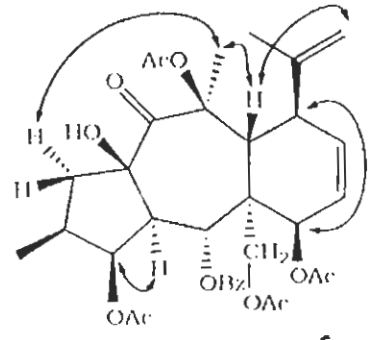


Figure 2.4: NOESY ^{1}H-^{1}H correlation[5] for decipinone (**79**).

2.1.2.3 X-Ray Structure Analysis of decipinone (79)

In order to firmly establish the structure of the molecule, the molecule was examined by X-ray crystallogrpahy which confirmed the structure of **79** as a tricyclic diterpene .. The crystal structure of **79** is shown in Fig. 2.5. However, the absolute configuration of **79** could not be established due to the poor quality of the crystals (for details see experimental section).

2.1.2.4 Proposed Biogenesis of decipinone (79)

According to Rentzea et. al. (64) such as the tetracyclic diterpenoids, (myrsinol esters, might be formed through the nucleophilic attack of epoxide oxygen of a 6,17-epoxy-lathyrol derivative (Scheme-1.19, page 40 of this thesis) which includes the construction of a three membered ring during that pathway. The structure of decipinone, which can be considered as a derivative of myrsinol with opened saturated furan ring, led us to suggest a different biogenesis for the formation of decipinone in which all the strained rings are opened.

According to Scheme 2.2, it is suggested that the carbon skeleton of decipinone (**79**) can be produced from a 6,17-epoxy-lathyrol derivative (**83**) (86). Ring closure between position C-6 and C-12 followed by ring opening of the cyclopropane between C-9/C-10, acetylation of the hydroxyl groups, and finally dehyrogenation between C-10 and C-18 produces the desired structure **79**.

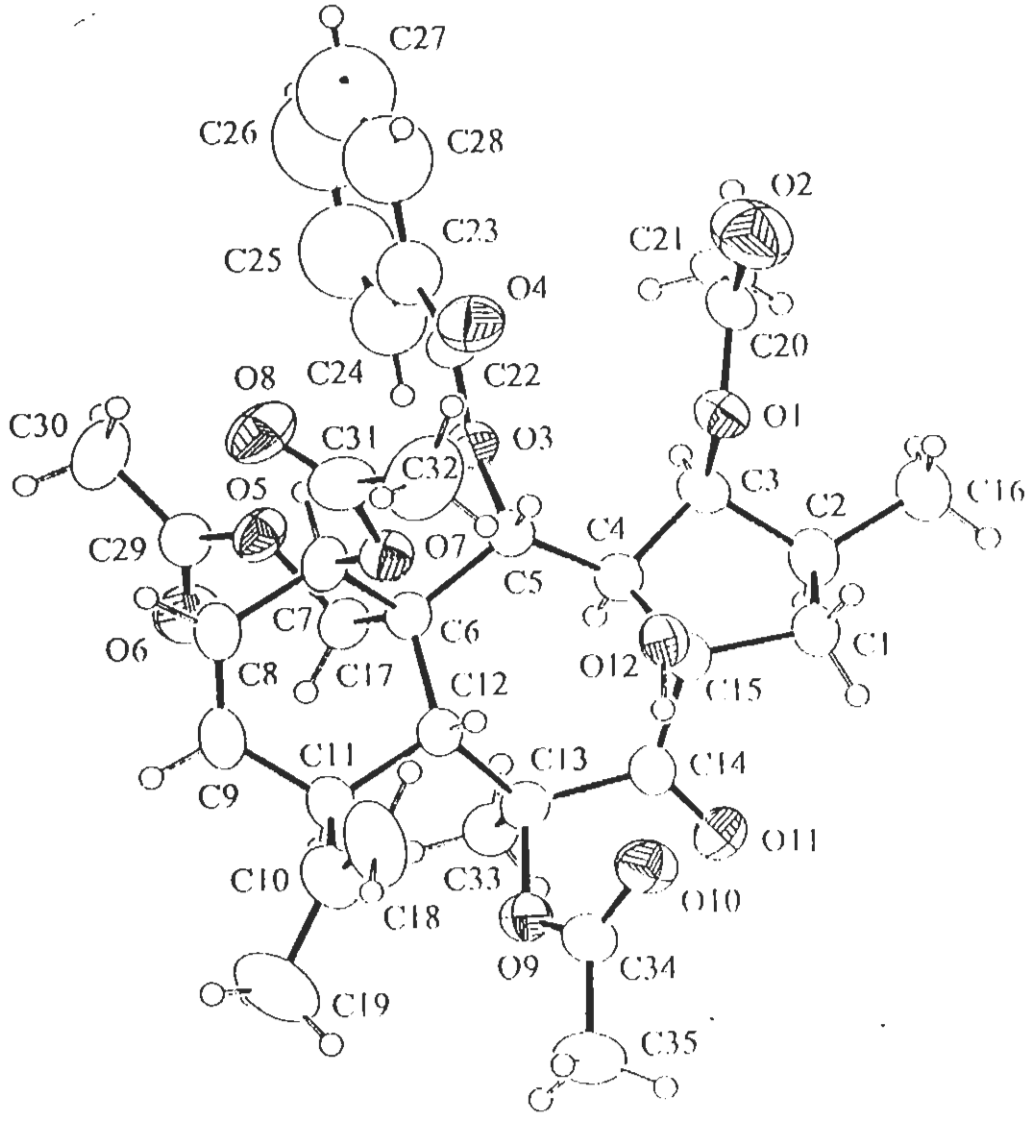
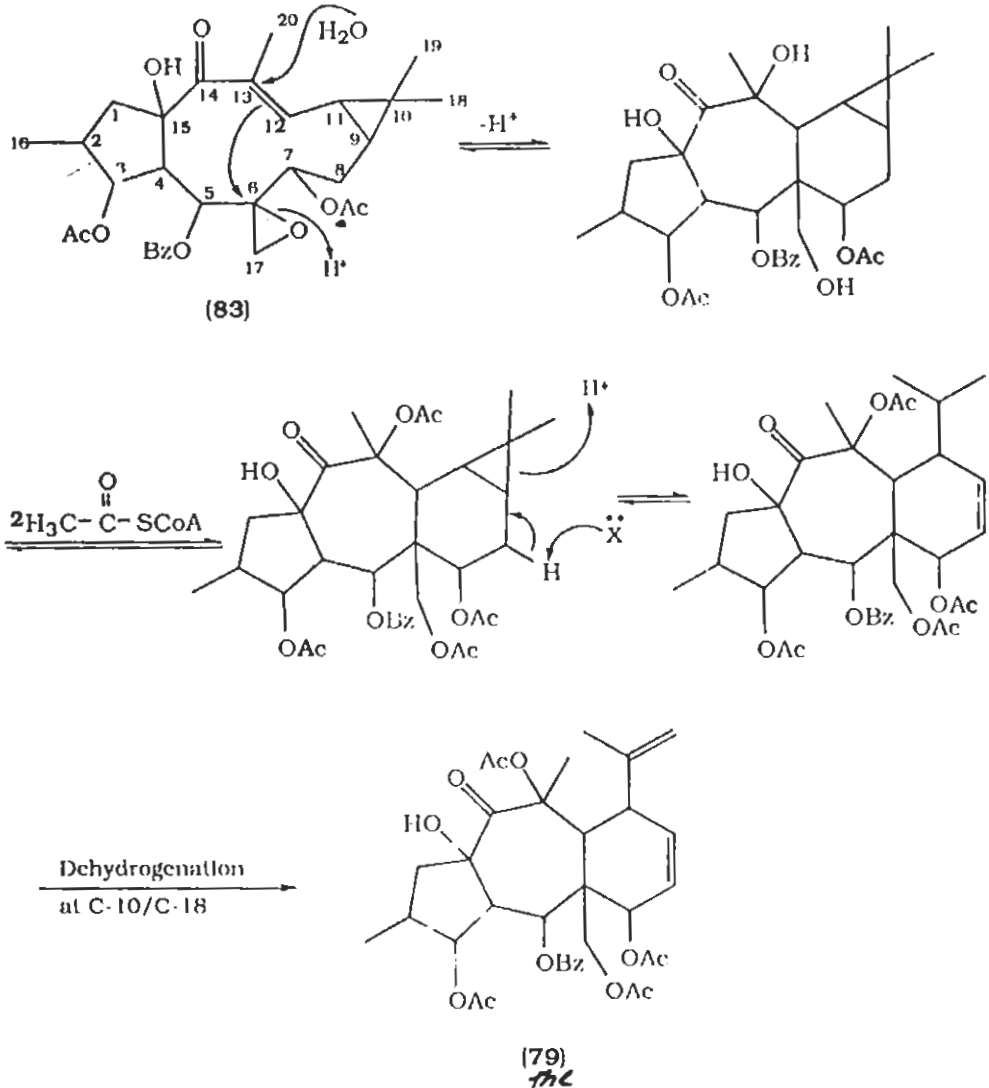


Figure 2. 5: The crystal structure of decipinone (79).

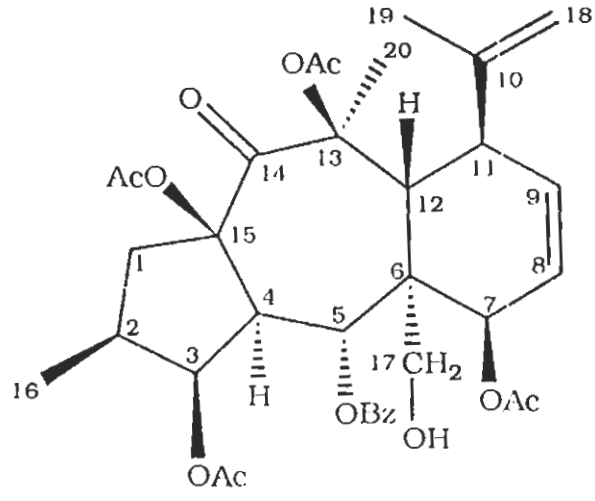


(83) (79)

Scheme-2.2: Proposed biogenesis for [illegible] of decipinone (**79**), from a 6,17-epoxy-lathyrol derivative (**83**).

2.1.3 Isodecipinone (84)

Isodecipinone (**84**) was obtained as a minor compound that was in equilibrium with decipinone. It was purified by preparative TLC (silica gel F_{254}) using chloroform: acetone (98 : 2) from decipinone.



(**84**)

2.1.3.1 Structure Elucidation of isodecipinone (84)

Isodecipinone (**84**) can be considered as an intramolecular transesterification product of decipinone (**79**) between positions C-15 and C-17. For isodecipinone (**84**) similar spectra, MS, IR, ^{1}H-NMR to decipinone (**79**) were obtained, but in ^{1}H-NMR (Table-2.2), there were some changes which included the upfield shift of H-12 at δ 3.54 (d, J=8.1 Hz) which had 0.53 ppm upfield shift in comparison to H-12 of decipinone (**79**), and conversion of H-17, 17' to AB signals at 4.09 (d, J=12 Hz) and 4.16 (d, J=12 Hz) which indicated a migration of an acetyl group from C-17 to free hydroxyl group at C-15. The upfield shift of H-12 in isodecipinone (**84**), [illegible] the effect of C-15 hydroxyl group lone pair electrons for deshielding of H-12. In

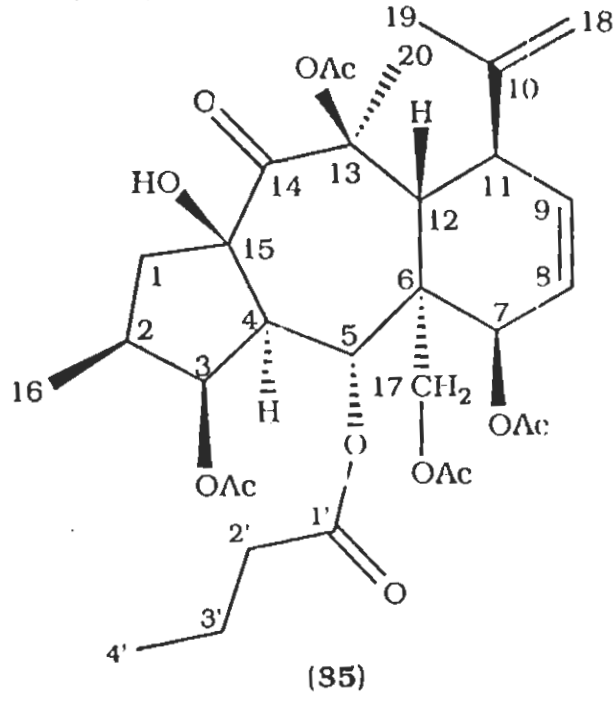
94 with acetylated C-15 . . . . the lone pair of electrons on oxygen due to bulky acetyl group, are not well oriented towards H-12. Also the brodening of the hydroxyl peak at 3480 cm^{-1} in the IR spectrum of **84** indicated the change location of the hydroxyl group in comparison to decipinone (**79**).

Table-2.2: ^{1}H-NMR spectral data of isodecipinone (**84**).

Position	^{1}H (J, Hz)
1α	3.34 dd (8.8, 14.9)
1β	1.79 dd (10.4, 14.9)
2	2.1 m
3	5.28 t (3.5)
4	2.61 dd (3.4, 11.6)
5	6.41 d (11.6)
7	4.67 d (6.4)
8	5.99 ddd (2.0, 6.4, 9.5)
9	5.74 dd (4.3, 9.5)
11	3.47 br d
12	3.54 d (8.1)
16	0.88 d (6.7)
17	4.09 d (12.0)
17'	4.16 d (12.0)
18	4.87 br d (2.0)
19	1.80 s
20	1.61 s
$OCOCH_3$	
	2.20 s
	2.15 s
	2.05 s
	1.89 s
Benzoyl	
2',6'	7.86 br dd (1.5, 10.0)
3',5'	7.39 br t (8.0)
4'	7.52 br tt (1.0, 8.5)
OH	4.97 s

2.1.4 Decipidone (85)

The second major diterpenoid, decipidone was obtained as white precipitate (from methanol) after preparative TLC (silica gel F_{254}) using chloroform-acetone (98:2).



(85)

2.1.4.1 Structure Elucidation of decipidone (85)

The molecular formula $C_{32}H_{44}O_{12}$ was assigned using HREIMS (obsd. 620.2789, calcd. 620.2833 for $C_{32}H_{44}O_{12}$). In the EIMS the base peak at *m/z* 71 indicated the presence of a C_3H_7CO fragment instead of benzoyl group. Its IR spectrum was very similar to that recorded for decipinone (**79**), but in this compound there was no indication of benzene ring signals. The absorption at λ_{max} (MeOH) 270 nm in the UV spectrum of decipinone (**79**), was not observed in the UV spectrum of **85** indicating the absence of the phenyl ring.

The ^{1}H and ^{13}C-NMR (Table-2.3) spectra of this compound were very similar to those recorded for **79** with the exception of some differences in chemical shifts, and also the peaks relating to benzoyl and butanoyl moiety. In the ^{1}H-NMR spectrum, instead of phenyl ring signals, a triplet at 0.91 (t, J=7.4 Hz, 3 x H-4') and signals for H-3' and H-2' at δ 1.5 and δ 2.1 were observed.

The ^{1}H-^{1}H vicinal correlations in butanoyl ester and the five membered ring were deduced from COSY 45° spectrum (Fig. 2.6), and further confirmed by double resonance spectroscopy. Irradiation of a multiplet at δ 2.1 collapsed the signals of Hα-1 to a doublet (J=14.5 Hz), and Me-16 and Me-4' signals to two singlets, indicating the overlap between H-2 and H-3' signals.

The corresponding peaks for butanoyl fragment in ^{13}C-NMR (DEPT) at δ 13.7 (q, C-4'), 17.9 (t, C-3') and 36.0 (t, C-2') were deduced according to HMQC data (Table-2.3). The cross peaks between H-5 and H-2' with the butanoyl carbonyl carbon at δ 171.8 in HMBC suggested location of butanoate ester at position 5.

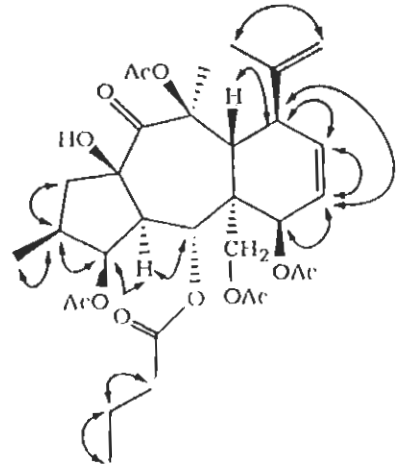


Figure 2.6: ^{1}H-^{1}H COSY correlations for decipidone (**85**).

Table-2.3 Spectral data of decipidone (85)*.

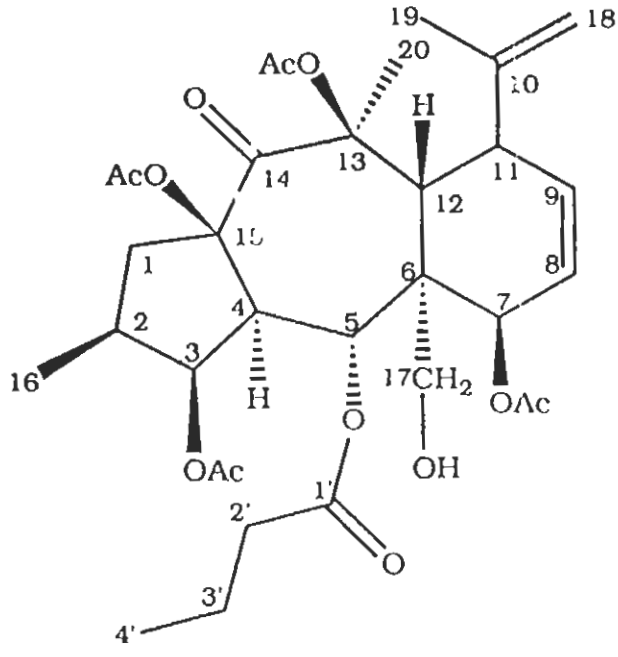
Position	^1H	^{13}C*	HMBC Correlations
1α	3.15 dd (9.5, 14.5)	45.8 (t)	C-2, C-3, C-4, C-14, C-15
1β	1.55 m		
2	2.10 m	37.6 (d)	
3	5.26 t (3.4)	79.6 (d)	$OCOCH_3$, C-1, C-15
4	2.30 dd (3.4,11.7)	54.1 (d)	OC-3, C-5, C-14, C-15
5	6.07 d (11.7)	70.1 (d)	C-1, C-2, C-4, C-6
6	-	48.1 (s)	
7	4.80 d (6.4)	67.8 (d)	$COCH_3$, C-6, C-8, C-9
8	5.99 ddd (1.9, 6.4, 9.6)	122.1 (d)	C-6, C-7, C-9
9	5.75 dd (4.6, 9.6)	133.5 (d)	C-6, C-7, C-11
10	-	147.0 (s)	
11	3.32 br t (6.0)	45.9 (d)	C-8, C-9, C-10, C-12, C-13, C-18, C-19
12	3.88 d (8.0)	41.8 (d)	C-5, C-6, C-10, C-11, C-13, C-17, C-19
13[a]	-	86.8 (s)	
14	-	205.9 (s)	
15[a]	-	86.9 (s)	
16	0.92 d (6.7)	14.4 (q)	C-1,C-2, C-3
17	3.90 d (12.0)	61.8 (t)	$OCOCH_3$, C-5, C-6, C-7, C-12
17'	4.21 d (12.0)		$OCOCH_3$, C-6, C-7
18	4.86 br s	113.0 (t)	
18'	4.87 br s		
19	1.76 s	19.7 (q)	C-10, C-11, C-18
20	1.64 s	22.6 (q)	C-12, C-13, C-14
$OCOCH_3$[b]			
	2.09 s	21.1 (q)	
	2.03 s	21.0 (q)	
	2.01 s	20.9 (q)	
	1.93 s	20.7 (q)	
$OCOCH_3$[b]			
	-	170.3 (s)	
	-	170.1 (s)	
	-	169.9 (s)	
	-	169.9 (s)	
$OCOC_3H_7$			
1'	-	171.8 (s)	
2'	2.10 m	36.0 (t)	
3'	1.50 m	17.9 (t)	C-2'
4'	0.91 t (7.4)	13.7 (q)	C-3', C-2'
OH	3.82 s		

* The ^1H-^{13}C connectivities and ^{13}C multiplicities were deduced according to HMQC and DEPT experiments.

[a,b] The assignments may be interchanged.

2.1.5 Isodecipidone (86)

Isodecipidone (86) was also obtained as a minor compound



(86)

2.1.5.1 Structure Elucidation of isodecipidone (86)

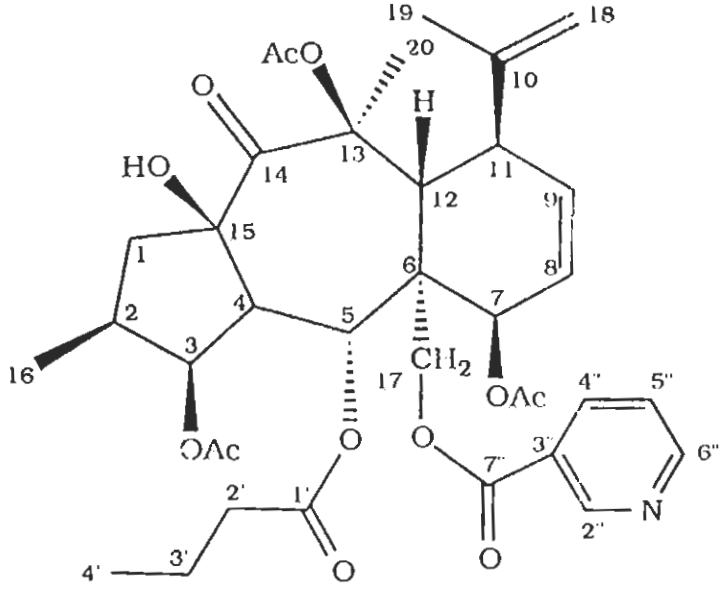
Decipidone (85) and isodecipidone (86), like the two previous compounds can be converted to each other in an intramolecular transesterification reaction between positions C-15 and C-17. The CIMS spectrum showed a molecular ion at *m/z* 621 $[M+1]^+$ indicating that 86 is an isomer of 85. In the the ^{1}H-NMR spectrum of 86 (Table 2.4) the same differences which were observed in the case of 84 led to proposal of the structure, as 86.

Table 2.4: ^{1}H-NMR spectral data of isodecipidone (**86**).

Position	^{1}H
1α	3.34 dd (8.8, 14.9)
2	2.1 m
3	5.25 t (3.6)
4	2.53 dd (11.6, 3.2)
5	6.14 d (11.6)
7	4.59 d (6.4)
8	5.99 m
9	5.76 br d (9.6)
11	3.47 m
12	3.63 d (9.7)
16	0.90 d (6.8)
17	3.91 d (12)
17'	4.07 d (12)
18	4.96 br s
18'	4.86 br s
19	1.78 s
20	1.56 s
$OCOC_3H_7$	
4'	0.93 d (7.2)
$OCOCH_3$	
	2.16 s
	2.08 s
	2.04 s
	1.98 s

2.1.6 Decipinone A (87)

Decipinone A (**87**) was obtained as a white powder (11 mg) by preparative TLC (silica gel F_{254}) using chloroform : acetone (92:8). **87** can be considered as the 17-O-nicotinoyl derivative of decipidone (**85**).



(87)

2.1.6.1 Structure Elucidation of decipinone A (87)

The IR spectrum showed characteristic peaks for hydroxyl (3500 cm^{-1}), carbonyl (1730 cm^{-1}) and unsaturation (1580 cm^{-1}). The UV spectrum showed an absorption at 260 nm indicating the presence of a nicotinoyl ester (87). The molecular formula was assigned as $C_{36}H_{45}O_{12}N$ on the basis of HREIMS (obsd. 683.2919, calcd. 683.2942). In the CI mass spectrum the molecular ion at m/z 683 and the peaks at m/z 623 $[M-HOAc]^+$ and 563 $[M-2 \times HOAc]^+$ were predominant.

In the EI mass spectrum the presence of ions at *m/z* 71 $[COC_3H_7]^+$ and 124 $[C_5H_4NCO]^+$ suggested that the molecule contains both butanoyl and nicotinoyl moieties.

The ^{1}H-NMR spectrum (in $CDCl_3$), (Table-2.5) exhibited three oxymethine protons at δ 5.23 (t, *J*=3.5 Hz, H-3), 6.07 (d, J=11.7 Hz, H-5), 5.02 (d, J=6.4 Hz, H-7), and one oxymethylene as a pair of doublets at δ 4.10 (d, J=12.2 Hz, H-17) and 4.66 (d, J=12.2 Hz, H-17'). The signals at 9.15 (br d, J=1.5 Hz, H-2"), 8.20 (dt, J=2.0, 8.0 Hz, H-4") 7.39 (dd, J=4.9, 8.0 Hz, H-5") and 8.78 (dd, J=1.5, 4.9 Hz, H-6") suggested the presence of a nicotinoyl moiety (4, 88). In addition to four methyl signals related to three acetyl groups at 2.07 (s), 2.02 (s), 1.94 (s) and one butanoyl methyl at δ 0.67 (t, J=7.4 Hz), one secondary and two tertiary methyls at δ 0.91 (d, J=6.8 Hz), 1.79 (s) and 1.67 (s) were recorded for H-16, H-19, and H-20, respectively. The other signals were more or less similar to those obtained for decipidone (**85**).

The ^{13}C-NMR spectra (Table-2.5, BB and DEPT) displayed 35 signals due to 36 carbons, including seven methyls in which two were overlapped, thirteen CH, five CH_2, and eleven quaternary carbons. The correlations between the ^{1}H and ^{13}C resonances were deduced through a HMQC experiment (Table-2.5). Except for the signals due to nicotinoyl group at 147.8 (d, C-2"), 127.4 (s, C-3"), 140.0 (d, C-4"), 124.9 (d, C-5"), 150.3 (d, C-6") and 163.1 (s, C-7") (88) there were no drastic changes in the ^{13}C-NMR chemical shift values of decipinone A (**87**) in comparison to the previous diterpenoids.

The relative position of the ester groups were determined by detection of cross peaks between H-3 and H-7 with the acetyl carbonyl groups at about δ 170, between H-17, 17' and the carbonyl of the nicotinoyl group at δ 163.1, and also the cross peaks between H-5 and H-2' with the carbonyl group of the butanoyl ester at δ 171.7 from the HMBC experiment (Table-2.5) The relative stereochemistry of **87** was deduced from coupling constants of the protons in the ^{1}H-NMR spectrum, as well as the cross peaks between H-5 and H-12 and H-12 and H-19 in the NOESY spectrum.

Table-2.5: Spectral data of decipinone A (87).

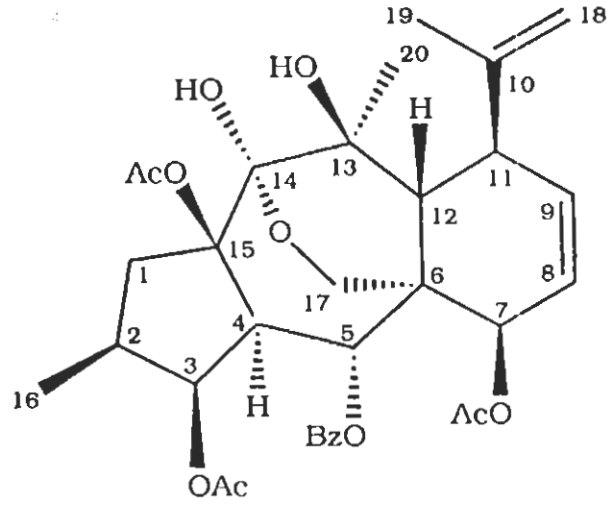
Position	^1H	^{13}C*	HMBC Correlations
1α	3.16 dd (9.7, 14.5)	46.0 (t)	C-14, C-15
1β	1.57 dd (10.5, 14.5)		
2	2.2 m	37.7 (d)	
3	5.23 t (3.5)	79.7 (d)	$OCOCH_3$, C-1, C-5, C-15
4	2.39 dd (3.3, 11.7)	54.8 (d)	
5	6.07 d (11.7)	70.4 (d)	$OCOC_3H_7$, C-4, C-6, C-7
6	-	48.4 (s)	
7	5.02 d (6.4)	67.3 (d)	$OCOCH_3$, C-6, C-8, C-9, C-12
8	6.06 m	122.2 (d)	
9	5.80 dd (4.7, 9.5)	136.8 (d)	
10		146.6 (s)	
11	3.40 br. t (7.0)	45.7 (d)	C-9, C-10, C-13
12	3.87 d (7.7)	42.1 (d)	C-6, C-10, C-13
13	-	87.2 (s)	
14	-	206.7 (s)	
15	-	86.6 (s)	
16	0.91 d (6.8)	14.5 (q)	C-1, C-2, C-3
17	4.10 d (12.2)	62.9 (t)	C-7", C-5, C-7
17'	4.66 d (12.2)		C-7
18	4.88 br s	113.4 (t)	C-10, C-19
18'	4.89 br s		
19	1.79 s	19.6 (q)	C-10, C-18
20	1.67 s	22.6 (q)	C-12 C-13
Acetyl**			
	1.94 s	170.3(21.1) (s,q)	
	2.02 s	170.1(21.1)(s,q)	
	2.07 s	170.0 (20.6)(s,q)	
Butanoyl			
1'	-	171.7 (s)	
2'	1.8 m	35.9 (t)	$OCOC_3H_7$
3'	1.34 m	17.8 (t)	$OCOC_3H_7$
4'	0.67 t (7.4)	13.6 (q)	C-2', C-3'
Nicotinoyl			
2"	9.15 br d (1.5)	147.8 (d)	
3"	-	127.4 (s)	
4"	8.20 dt (2.0, 8.0)	140.0 (d)	
5"	7.39 dd (4.9, 8.0)	124.9 (d)	
6"	8.78 dd (1.5, 4.9)	150.3 (d)	
7"	-	163.1 (s)	

*The ^1H-^{13}C connectivities and ^{13}C-multiplicities were deduced according to HMQC and DEPT experiments.

**The assignments may be interchanged.

2.1.7 Decipinol ester A (88)

Decipinol ester A (**88**) was obtained as a white powder (11 mg), and purified by preparative TLC (silica gel F_{254}) using chloroform-acetone (92:8).



(88)

2.1.7.1 Structure Elucidation of decipinol ester A (88)

The IR spectrum showed absorptions at 3450, 1730 and 1640 cm^{-1} indicating the presence of hydroxyl, carbonyl and unsaturation in the molecule, respectively. Its molecular formula was assigned on the basis of CIMS as $C_{33}H_{40}O_{11}$, *m/z* 612$[M]^+$. In the EIMS the ions at *m/z* 594 $[M-H_2O]^+$, 552 $[M-HOAc]^+$ and 492 [M-2 X HOAc]; and the base peak at *m/z* 105 $[C_6H_5CO]^+$ indicated the presence of hydroxyl, acetate and benzoate functionalities in the molecule. The UV spectrum showed absorptions at 199.0, 227.6 and 270 nm, indicating the presence of a phenyl moiety in the molecuole.

The ^{1}H-NMR spectrum (Table 2.6) was similar to that recorded for decipinone (**79**) except the signals of ester moieties and also differences resulting from closure of the hemiacetal ring between the C-14 carbonyl and hydroxyl groups. The long range coupling between H-17 at δ 3.77 (dd, J=1.3, 11.3 Hz) and H-5 at δ 5.68 (dd, J=1.3, 10.8 Hz), upfield shift of H-12 at δ 2.89 (br s) and of H-1 at δ 2.38 (dd, J=10.9, 14.7 Hz), are the consequences of the formation of a six membered hemiacetal ring in **88**. These upfield shifts indicate the lack of an anisotropic effect of the C-14 carbonyl group as well as C-15 hydroxyl group which is acetylated. The olefinic signals of H-8 and H-9 were changed into broad triplets at δ 6.28 (J=6.7 Hz) and 6.36 (J=7.2 Hz) respectively, indicating the effect of the new conformation of the six membered ring (C), on the multiplicities of the signals.

In the ^{13}C-NMR spectra (in CD_3OD, 125 MHz), the quaternary signals at δ 77.8 (C-13), 100.1 (C-14) and 98.0 (C-15) together with upfield shift of H-20 at δ 1.34 in ^{1}H-NMR led us to conclude that the hemiacetal bearing carbon atom, the acetate unit, and the free hydroxyl groups are located at C-14, C-15 and C-13, respectively. The other difference observed in the ^{13}C-NMR spectrum (in $CDCl_3$) of **88** the upfield shifts of C-1, C-2, C-3 and C-11 at δ 38.3, 33.2, 72.9, and 40.7, respectively, in comparison to the previously isolated diterpenoids.

The connectivities between H-17 and C-14 and H-20 and C-13 in the HMBC spectrum (Table 2.6) confirmed the structure of **88**. The relative positions of the ester moieties were established from HMBC spectrum at positions C-3, C-5 and C-7, and the downfield shift of C-15 and upfield shift of C-13 were indicative of the acetyl group at C-15 and an

hydroxyl group at C-13. In the NOESY spectrum correlations of H-17 with H-7 and H-20 and H-12 with H-5 and H-19 confirmed the stereostructure of **88**.

In order to establish the presence of C-14 quaternary carbon atom in ^{13}C-NMR spectrum and the correlation between H-7 and the acetyl carbonyl signal in HMBC spectrum, **88** was subjected to NMR spectroscopic experiments in CD_3OD (Table 2.7).

Table-2.6: Spectral data of decipinol ester A (88)* (in $CDCl_3$).

Position	^1H	^{13}C*	HMBC Correlations
1α	2.38 dd (10.9, 14.7)	38.3 (t)	C-3, C-4, C-15, C-16
1β	2.47 br d (14.7)		-
2	2.56 m	33.2 (d)	-
3	5.29 dd (5.2, 7.3)	72.9 (d)	$OCOCH_3$, C-1, C-15
4	2.72 dd (5.2, 10.8)	54.5 (d)	C-3, C-5, C-15
5	5.68 dd (1.3, 10.8)	73.2 (d)	OCOPh, C-3, C-4, C-6, C-7, C-12
6	-	45.9 (s)	-
7	4.53 d (6.1)	66.7 (d)	C-6, C-8, C-9, C-12, C-17
8	6.28 br t (6.7)	127.4 (d)	C-7, C-11
9	6.36 br t (7.2)	137.3 (d)	C-7, C-11
10	-	147.3 (s)	
11	3.17 d (6.9)	40.7[a](d)	C-6, C-8, C-9, C-10, C-12, C-13, C-18
12	2.89 br s	40.9[a](d)	C-5, C-6, C-7, C-9, C-10, C-11, C-13, C-20
13	-	77.6 (s)	
14	-	98.6 (s)	
15	-	96.8 (s)	
16	0.87 d (6.7)	16.1 (q)	C-1, C-2, C-3
17	3.77 dd (1.3, 11.3)	66.5 (t)	C-5, C-6
17'	4.09 d (11.3)		C-5, C-6, C-12, C-14
18	4.55 br s	110.9 (t)	C-10, C-11, C-19
18'	4.80 br d (1.5)		C-11, C-19
19	1.91 s	22.2 (q)	C-10, C-11, C-18
20	1.34 s	22.2 (q)	C-12, C-13
$OCOCH_3$[b]			
	1.62 s	22.5 (q)	
	2.07 s	20.9 (q)	
	2.19 s	20.5 (q)	
$OCOCH_3$[b]			
	-	174.2 (s)	
	-	170.9 (s)	
	-	169.7 (s)	
Benzoyl			
1'	-	129.6 (s)	
2',6'	7.87 br dd(1.1, 8.1)	129.4 (d)	
3',5'	7.37 br t (8.0)	128.5 (d)	
4'	7.51 br tt(1.2, 7.4)	133.2 (d)	
7'	-	164.5 (s)	

* The ^1H-^{13}C connectivities and ^{13}C multiplicities were deduced according to HMQC and DEPT experiments.
[a,b]The assignments may be interchanged.

Table-2.7: Spectral data of decipinol ester A*(in CD_3OD).

Position	^1H	^{13}C	HMBC Correlations
1α	2.36 dd (11.1, 14.8)	39.3 (t)	C-3, C-4, C-15, C-16
1β	2.56 m		
2	2.56 m	34.3 (d)	
3	5.28 dd (5.3, 7.4)	74.8[a] (d)	$COCH_3$, C-1, C-15
4	2.85 dd (4.9, 10.8)	55.5 (d)	
5	5.70 dd (1.4, 10.8)	74.9[a] (d)	*COPh*, C-4, C-12, C-17
6	-	47.2 (s)	
7	4.53 d (6.4)	68.3 (d)	C-6, C-8, C-9, C-17
8	6.24 dd (6.4, 9.5)	128.1 (d)	C-7, C-11
9	6.40 dd (7.2, 9.5)	138.9 (d)	C-7, C-11, C-12
10	-	149.3 (s)	
11	3.21 d (7.2)	41.9[b] (d)	C-6, C-10, C-12,C-13
12	2.91 s	41.8[b] (d)	C-5, C-6, C-9, C-10, C-11, C-13, C-20
13	-	77.8 (s)	
14	-	100.1 (s)	
15	-	98.0 (s)	
16	0.87 d (7.4)	16.4 (q)	C-1,C-2,C-3
17	3.78 dd (1.6, 11.2)	67.1 (t)	C-5
17'	4.15 d (11.3)		C-5, C-6, C-12, C-14,
18	4.54 br s	111.0 (t)	C-10, C-11, C-19
18'	4.81 br q (1.5)		C-11, C-19
19	1.95 s	22.7[c] (q)	C-10, C-11
20	1.29 s	22.9[c] (q)	C-12, C-13, C-14
$OCOCH_3$			
	2.19 s	21.6 (q)	
	2.07 s	21.0 (q)	
	1.61 s	20.8 (q)	
$OCOCH_3$			
	-	174.9 (s)	
	-	173.4 (s)	
	-	171.4 (s)	
Benzoyl			
1'	-	130.9 (s)	
2',6'	7.93 dd (1.2, 8.3)	129.6 (d)	C-3', 5', C-4',C-7', C-1'
3',5'	7.44 dt (1.5, 8.2)	130.5 (d)	C-2, 6', C-1'
4'	7.58 tt (1.3, 7.5)	134.9 (d)	C-3',5'
7'	-	166.1 (s)	-

• The ^1H-^{13}C connectivities and ^{13}C multiplicities were deduced according to HMQC and DEPT experiments.

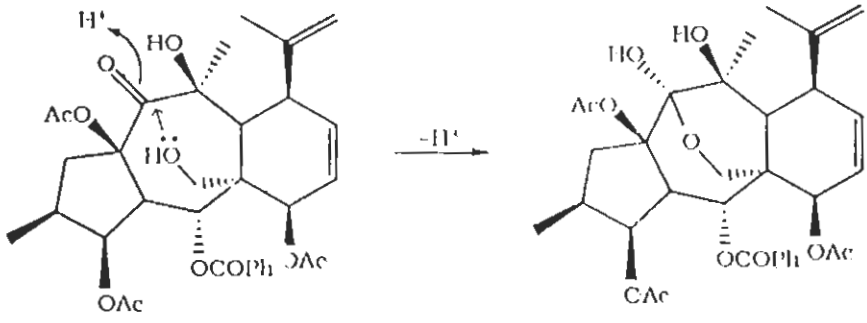
[a,b,c] The assignments may be interchanged.

2.1.7.2 X-Ray Structure Analysis of decipinol ester A (88)

The presence of a hemiacetal ring in the molecule (**88**) was confirmed by an X-ray crystallography experiment. The crystals of decipinone B (**88**) were grown from methanol, and a single colourless, plate-like crystal was subjected for the experiment (see the experimental section). The crystal structure of **88** is given in Fig. 2.7.

2.1.7.3 Proposed Biogenesis for decipinol ester A (88)

The presence of a six membered ring in **88** can be the consequence of hemiacetal formation between free hydroxyl at C-17 and the C-14 carbonyl group of an imaginary 17-deacetoxy decipinone (**89**) precursor.



(89) (88)

Scheme-2.3: Biogenetic pathway for decipinol ester A (**88**).

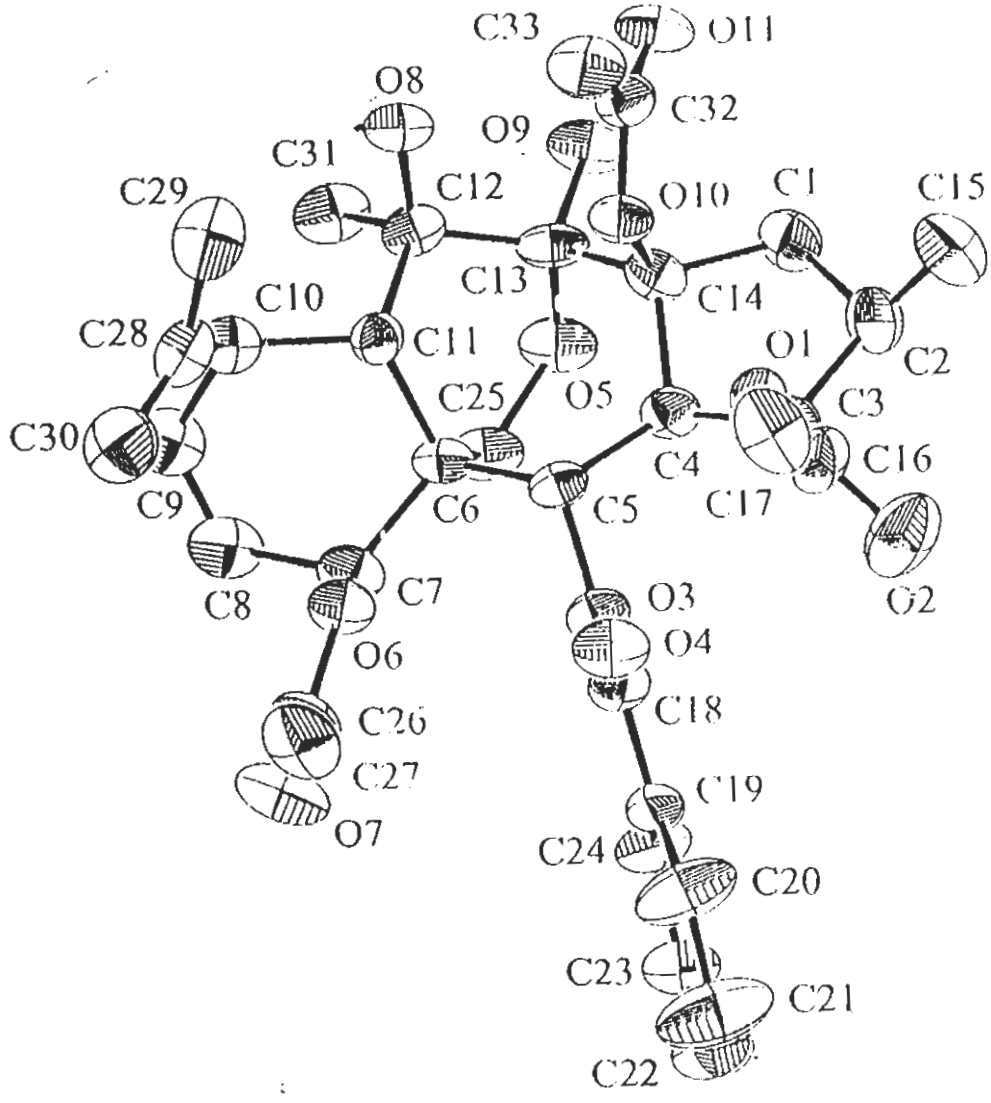
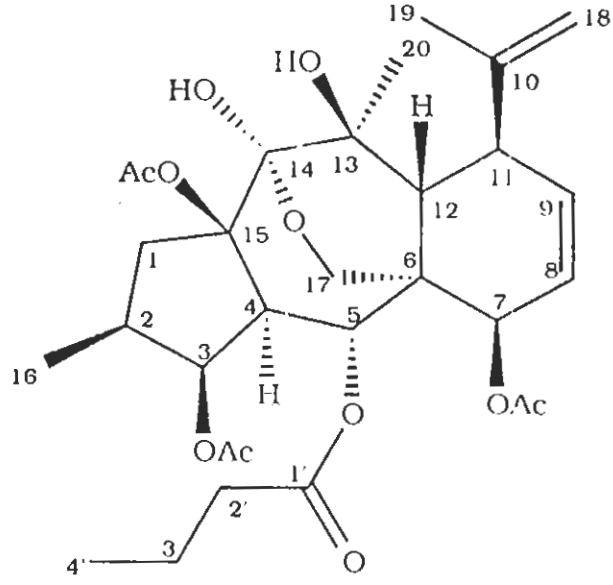


Figure 2. 7: The crystal structure of decipinol ester A.

It is important to mention here that, the absolute configuration of the compound could not be determined. The Flack parameter was 0.1 (7) for the structure reported here. Its value for the opposite configuration (structure 88) was 0.5 (8).

2.1.8 Decipinol ester B (90)

Decipinol ester B (**90**) is the 5-O-butanoyl ester of **88**, and obtained as colourless crystals (methanol). The compound was purified by repeated column chromatography (see experimental section).



(**90**)

2.1.8.1 Structure Elucidation of decipinol ester B (90)

The molecular formula was assigned on the basis of EIMS. In the EIMS the ions at *m/z* 578 ($C_{30}H_{42}O_{11}$) $[M]^+$ (0.4), 560 $[M-H_2O]^+$ (1), 518 [M-HOAc]$^+$ (1), 490 $[M-C_3H_7CO_2H]^+$, 458 [M-2xHOAc]$^+$ (2) and 71 $[C_3H_7CO]^+$ (100) indicated the presence of hydroxyl, acetate and butanoate moieties in the molecule. IR spectrum showed absorptions for carbonyl (1712, 1728, 1736 cm^{-1}) and hydroxyl (3450 cm^{-1}). The UV spectrum exhibited a maximum at 203 nm indicating the absence of phenyl group in the molecule.

The ^{1}H-NMR spectrum (Table 2.8) was very similar to that of **88** except for the benzoyl moiety which was replaced by butanoyl signals at δ 0.90 (t, J=7.4 Hz, H-4'), 1.9 (m, H-3') and 2.0 (m, H-2'). The ^{13}C-NMR spectra (BB and DEPT) showed four signals at 171.3 (s, C-1'), 36.1 (t, C-2'), 18.3 (t, C-3') and 13.7 (q, C-4') for a butanoyl group instead of the phenyl in **88**. The other signals were close in their chemical shifts to those recorded for **88**. The correlation between H-5 and H-2' with the carbonyl of the butanoyl ester at δ 171.7 determined the position of the butanoyl moiety at C-5.

Table-2.8: Spectral data of decipinol ester B (90)*.

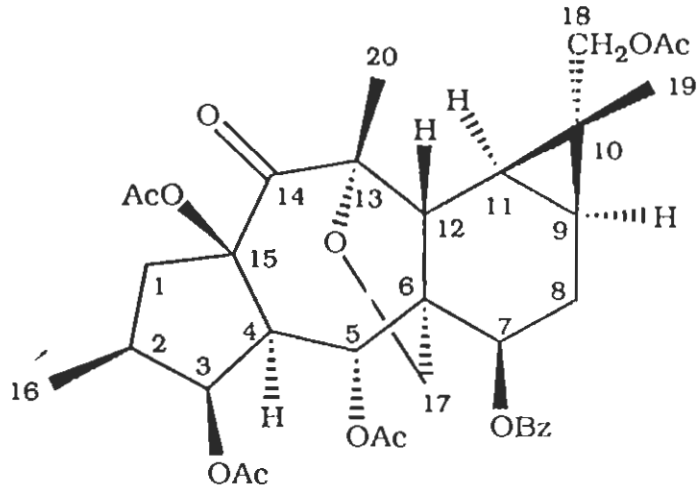
Position	^1H	^{13}C	HMBC Correlations
1α	2.34 dd (10.9,14.7)	38.3 (t)	C-3,C-4,C-15, C-16
1β	2.46 br d (14.1)		
2	2.5 m	33.2 (d)	
3	5.27 dd(5.5, 7.4)	72.9 (d)	C-5, C-15
4	2.56 dd(5.5, 10.7)	54.5 (d)	
5	5.43 dd (1.1, 10.7)	72.3 (d)	C-1', C-7
6	-	45.9 (s)	
7	4.47 br d(5.6)	66.2 (d)	
8	6.26 br t (6.5)	127.3 (d)	
9	6.37 br t (7.4)	137.6 (d)	
10	-	147.6 (s)	
11	3.15 d (6.9)	40.7[a] (d)	C-6, C-8, C-9, C-12, C-13
12	2.82 br s	40.9[a] (d)	C-5, C-6, C-9, C-10, C-11, C-13
13	-	77.6 (s)	
14	-	98.6 (s)	
15	-	96.8 (s)	
16	0.87 d (7.1)	16.1 (q)	C-1, C-2, C-3
17	3.67 br d (10.7)	66.2 (t)	C-5, C-6, C-12, C-14
17'	3.90 d(11.3)		
18	4.54 br s	110.6 (t)	C-12, C-19
18'	4.79 d (1.5)		
19	1.89 s	22.2[b] (q)	C-10, C-11, C-18
20	1.31 s	22.5[b] (q)	C-12, C-13, C-14
$OCOCH_3$[c]			
	1.79 s	22.16 [b] (q)	
	2.04 s	20.8 (q)	
	2.11 s	20.8 (q)	
$OCOCH_3$			
	-	174.2 (s)	
	-	171.0 (s)	
	-	170.2 (s)	
Butanoyl			
1'	-	171.3 (s)	
2'	2.0 m	36.1 (t)	C-1'
3'	1.9 m	18.3 (t)	C-1'
4'	0.90 t (7.4)	13.7 (q)	C-2', C-3'

* The ^1H -^{13}C connectivities and ^{13}C multiplicities were deduced according to HMQC and DEPT experiments.

[a,b,c] The assignments may be interchanged.

2.1.9 Karajinone A (91)

Two pentacyclic diterpenoids, related to the lathyrane skeleton were isolated from this plant and were named karajinone A and B, the name was derived from the collection place of the plant, Karaj, a city in Iran. It was obtained as a white precipitate (methanol) and purified by preparative TLC (silica gel F_{254}) using chloroform-acetone (92:8).



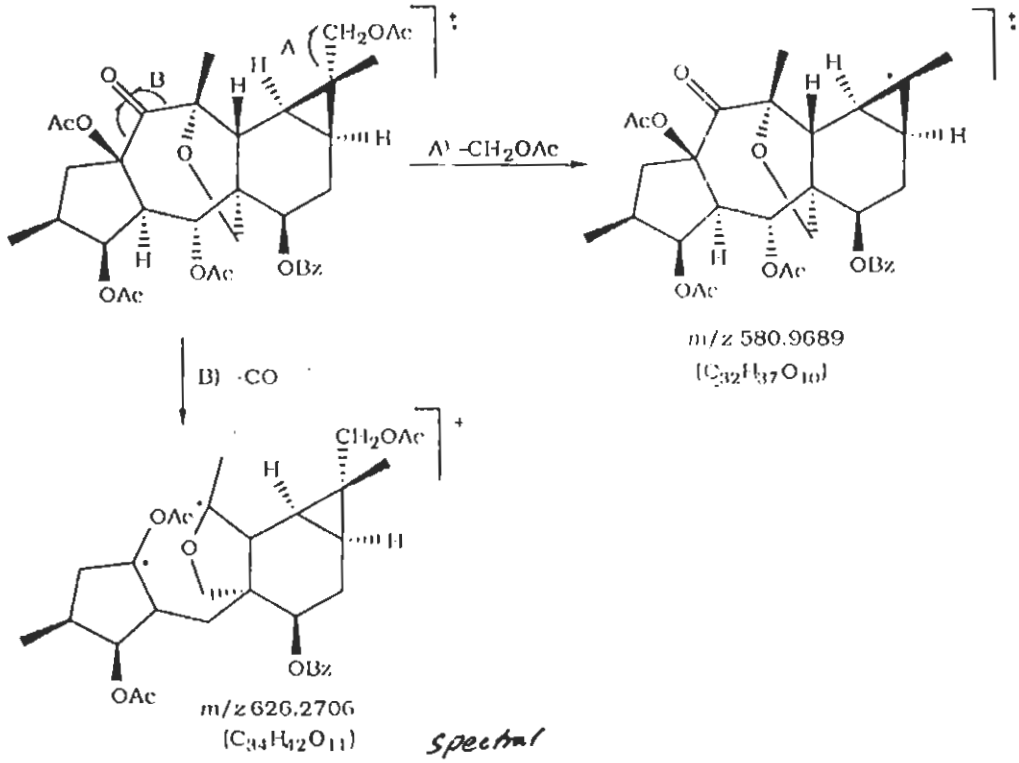
(91)

2.1.9.1 Structure Elucidation of karajinone A (91)

The molecular formula was assigned as $C_{35}H_{42}O_{12}$ on the basis of the CI mass spectrum which showed the $[M]^+$ peak at m/z 654 (10%) and two peaks at m/z 594 $[M\text{-}HOAc]^+$ (100%) and 534 $[M\text{-}2 \times HOAc]^+$ (98%). The peaks at m/z 105 (94%) $[C_6H_5CO]^+$ in the EIMS spectrum indicated the presence of a benzoate ester in the molecule. Similarly the IR (1740, 1730, 1605 and 980 cm^{-1}) spectral data confirmed the carbonyl and phenyl functionalities.

The proposed mass fragmenation pattern for this compound is illustrated in Scheme-2.4. The molecular ion with loss of CO, produced the ion at *m/z* 626, also elimination of CH_2OAc from M+ can result in the ion at *m/z* 581.

In the ^{1}H-NMR spectrum (in $CDCl_3$, 500 MHz, Table 2.9) of **91**, besides the signals of the first spin system, H-1 to H-5, which are similar to the decipinone type compounds, a doublet of doublets at δ 3.97 (dd, *J*=1.0, 9.7 Hz, H-17), and a doublet at δ 5.93 (dd, *J*=0.8, 10.9 Hz, H-5) were observed. The long range W coupling (*J*=1 Hz) between H-5 and H-17, together with a geminal coupling with a small J value of *J*=9.7 Hz, between H-17 and H-17', established a saturated furan ring through C-17 and C-13 which was also observed in the ^{1}H-NMR spectrum of myrsinol esters (4, 54).



Scheme-2.4: Proposed mass fragmentation pattern for karajinone A (**91**).

The other charac teristic peaks in the ^{1}H-NMR spectrum of **91** were as follow:

A tertiary methyl at δ 1.17 (s, H-19), a pair of doublets at δ 3.78 (d, J=11.2 Hz, H-18) and 3.83 (d, J=11.2 Hz, H-18'), the upfield shift of H-12 towards 2.82 (br d, J=4.5 Hz), a doublet of doublets at δ 4.94 (J=4.4, 7.2 Hz, H-7), and three multiplets at δ 1.85, 2.2 and 1.13 due to H-8, H-8', and H-9, respectively and finally, a triplet at δ 1.18 (J=7.0 Hz, H-11). The correlation between the vicinal protons in the H-7 to H-12 spin system were deduced through a ^{1}H-^{1}H COSY 45° spectroscopy experiment (Fig. 2.8).

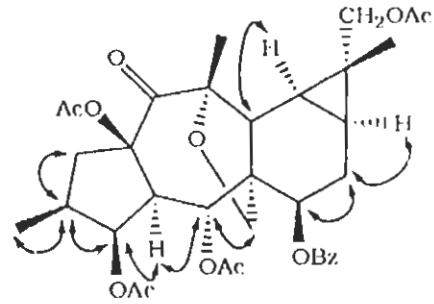
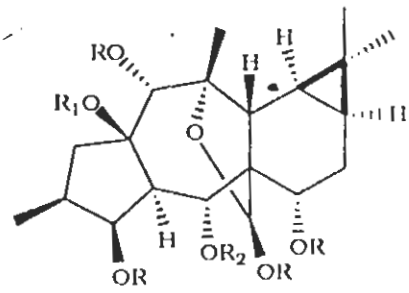


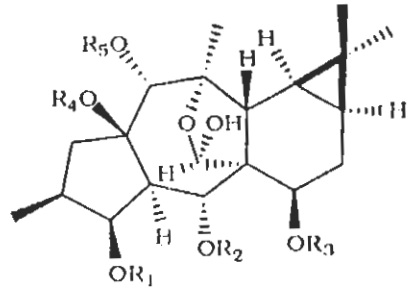
Figure 2.8: ^{1}H-^{1}H COSY 45° correlation for karajinone A.

The ^{13}C-NMR spectrum of **91** (Table-2.9), displayed six CH_3, four CH_2, eleven CH and eleven quaternary carbon atoms. The upfield signals at δ 19.72 (d, C-9); 22.12 (s, C-10) and 15.49 (d, C-11), suggested a cyclopropane ring (89-92) which was substituted at C-10 with a methyl group [δ 11.98 (q, C-19)] and an acetoxy methylene [δ 73.6 (t, C-18)]. The presence of an extra triplet at δ 23.3 (C-8) is another important difference compared with the decipinone-type compounds. The assignment of the carbon skeleton and the ester group locations were established by the HMBC spectrum (Table 2.9), as well as by comparison of the ^{1}H and ^{13}C-NMR chemical shifts and coupling constants observed for the myrsinol esters,

(82) (4, 54), aleppicatines A and B **(92)** (91), and the pentacyclic diterpenes, euphoppins A-D **(93)** (92) isolated from *E.aleppica*.



(92)



(93)

The stereochemistry of the compound was determined by comparison of the ^{1}H-NMR coupling constants of **91** with those recorded for myrsinol esters and the diterpene esters isolated from *E. aleppica* (4, 54, 91, 92) with similar structures, as well as by NOE difference spectroscopy and NOESY spectra. The NOESY cross peaks between H-3 and H-4, H-5 and H-12, and H-12 and H-19 established that H-5, H-12 and H-19 must be located on one face of the molecule. In NOE difference spectroscopy, irradiation of H-18 gave significant enhancement to the H-11, H-19 (2.3%) and H-9 (3.7%) signals. Irradiation of H-17 enhanced H-7 (2.3%) and H-17' (1.8%) which confirmed H-7 as α. Finally, irradiation of H-19 enhanced H-2',6' and (1.3%), H-5 (1.8%), H-12 (3.7%) and H-18' (1.8%) signals which confirmed the position of the benzoate moiety, as well as the configurations of H-5, H-9, H-11 and H-12.

Table-2.9: Spectral data of Karajinone A (**91**)*.

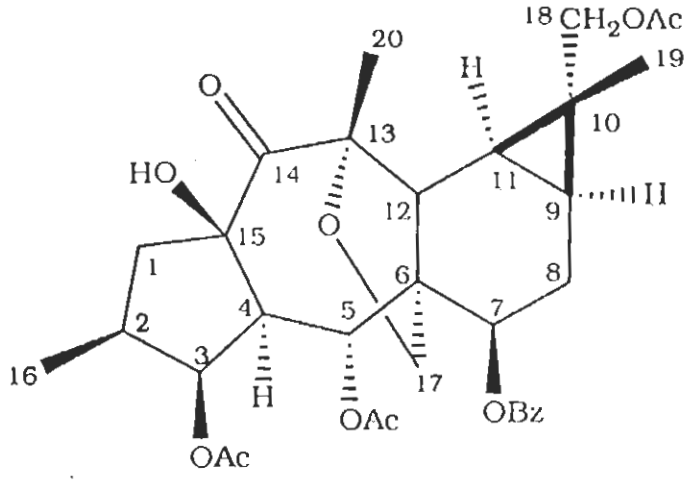
Position	^1H	^{13}C	HMBC Correlations
1α	3.41 dd (9.5, 14.9)	42.16 (t)	C-2, C-3, C-14
1β	1.47 dd (10.0,14.9)		
2	2.1 m	36.15 (d)	
3	5.16 t (3.9)	77.60 (d)	C-1, C-15
4	2.48 dd (3.6, 10.9)	53.32 (d)	C-3, C-5
5	5.93 dd (0.8, 10.9)	68.30 (d)	$OCOCH_3$, C-3, C-6, C-17
6	-	54.18 (s)	
7	4.94 dd (4.4, 7.2)	73.46 (d)	
8	1.85 m	23.34 (t)	
8'	2.2 m		
9	1.13 m	19.72 (d)	
10	-	22.12 (s)	
11	1.18 t (7.0)	15.49 (d)	
12	2.82 br d (4.5)	39.19 (d)	C-5, C-7, C-10, C-11, C-17
13	-	89.90 (s)	
14	-	201.40 (s)	
15	-	88.94 (s)	
16	0.87 d (6.8)	14.53 (d)	C-1, C-2, C-3
17	3.97 dd (1.0, 9.7)	71.65 (t)	C-5, C-7, C-13
17'	4.17 d (9.7)		C-5, C-6, C-12, C-13
18	3.78 d (11.2)	73.60 (t)	$OCOCH_3$, C-9, C-10, C-11, C-19
18'	3.83 d (11.2)		
19	1.17 s	11.98(q)	C-9, C-10, C-11, C-18
20	1.55 s[a]	20.49 (q)	C-12, C-14
$OCOCH_3$[b]			
	1.54 s [a]	20.49 (q)	
	1.96 s	20.84 (q)	
	2.03 s	21.01 (q)	
	2.18 s	21.56 (q)	
$OCOCH_3$			
	-	169.14 (s)	
	-	170.69 (s)	
	-	170.95 (s)	
	-	169.65 (s)	
Benzoyl			
1'	-	130.40 (s)	
2',6'	7.95 br dd (1.0, 8.0)	129.71 (d)	
3',5'	7.41 br t (8.0)	128.30 (d)	
4'	7.54 br tt (1.0, 7.5)	133.11 (d)	
7'	-	166.01 (s)	

*The ^1H -^{13}C connectivities and ^{13}C multiplicities were deduced according to HMQC and DEPT experiments.

[a,b] The assignments may be interchanged.

2.1.10 Karajinone B (94)

Karajinone B (**94**) is a C-15 deacetoxy derivative of Karajinone A (**91**). It is a white powder which was purified by preparative TLC with the same solvent system as for Karajinone A.



(**94**)

2.1.10.1 Structure Elucidation of karajinone B (94)

Karajinone B (**94**) exhibited a molecular ion at *m/z* 612 (CIMS), suggesting the molecular formula $C_{33}H_{40}O_{11}$. The IR spectrum showed a broad peak at 3450 cm^{-1} indicating the presence of a hydroxyl group.

In the ^{1}H-NMR spectrum (in $CDCl_3$, 500 MHz, Table 2.10), there were three methyl singlets for acetyl groups at δ 2.05, 2.07 and 1.34 indicating this compound is the deacetyl derivative of **91**. The up field shift for the acetyl methyl as was mentioned earlier, might be due to the anisotropic effect of one of the other ester groups, e.g. the benzoate moiety. Despite the fact that only one acetyl was omitted from the parent molecule, the effect on the chemical shifts and values of the characteristic signals

were considerable, for instance the signals at δ 2.71 (H-1α), 0.98 (H-11), 1.39 (H-20) and one acetyl at δ 1.34 were obviously shifted towards upfield. In contrast, the signals at δ 1.72 (H-1β), and 3.08 (H-12) were shifted downfield which is due to the deshielding effect of the hydroxyl group at C-15. The coupling constant of 6.7 Hz between H-11 and H-12 indicated that the dihedral angle between them is near to 180° than that for the acetylated compound.

In the ^{13}C-NMR spectra (BB, DEPT,Table 2.10), C-15 resonated at δ 83.05 which was an upfield shift of δ 5.89 ppm in comparison to the acetylated compound, thereby confirming the position of the free hydroxyl group. The relative positions of the ester groups were deduced by the observations of cross peaks between H-5, H-18 and H-7 and the corresponding carbonyl esters at δ 169.97 and 169.8 for acetyl groups, and at δ 166.4 for the benzoyl moiety, respectively. The stereochemistry and vicinal correlations were further confirmed by the interpretation of NOESY, ^{1}H-^{1}H COSY and ^{1}H -NMR spectral data.

Table-2.10: Spectral data of karajinone B (94)*.

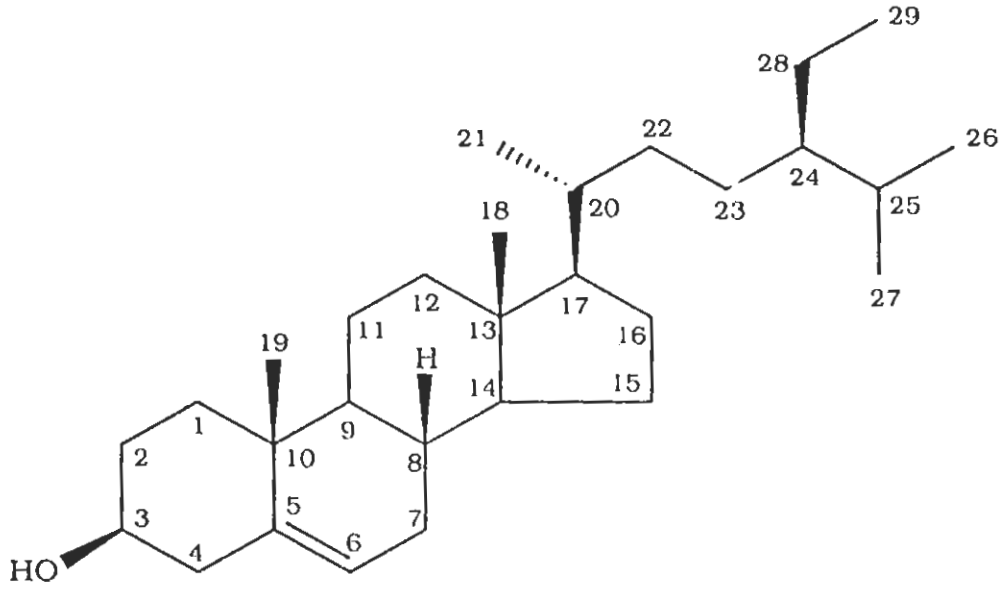
Position	^1H	^{13}C	HMBC Correlations
1α	2.71 dd (11.2,15.6)	46.29 (t)	C-3
1β	1.72 dd (7.7, 15.6)		C-15
2	2.1 m	34.07 (d)	
3	5.24 dd (3.7,4.8)	79.33 (d)	
4	2.99 dd (3.7,10.9)	52.40 (d)	C-5, C-15
5	5.74 dd (1.5, 10.9)	68.09 (d)	$OCOCH_3$, C-4, C-7
6	-	54.38 (s)	
7	4.88 dd (3.2, 10.7)	76.30 (d)	C-5, C-6, C-12, C-13, OCOPh
8	1.7 dd (8.0, 14.3)	24.22 (t)	
8'	2.1 m		
9	1.2 m	21.65 (d)	
10	-	23.76 (d)	
11	0.98 dd (6.7, 9.9)	14.81 (d)	
12	3.08 d (6.7)	41.06 (d)	C-5, C-6, C-7, C-10, C-11
13	-	88.06 (s)	
14	-	-	
15	-	83.05 (s)	
16	0.92 d (7.1)	15.41 (q)	C-1, C-2, C-3
17	4.04 dd (1.5, 9.9)	74.41 (t)	C-5, C-7
17'	4.46 d (9.9)		C-5, C-6, C-12, C-13
18	3.73 d (11.2)	73.28 (t)	C-9, C-10, C-11, $OCOCH_3$
18'	3.91 d (11.2)		$OCOCH_3$, C-10, C-11
19	1.13 s	11.63 (q)	C-9, C-10, C-11, C-18
20	1.39 s	19.25 (q)	C-12, C-13
$OCOCH_3$[a]			
	1.34 s	20.84 (q)	
	2.05 s	21.03 (q)	
	2.07 s	21.21 (q)	
$OCOCH_3$			
	-	169.97 (s)	
	-	169.80 (s)	
	-	169.80 (s)	
Benzoyl			
1'	-	-	
2',6'	7.95 br dd (1.3, 8.2)	129.90 (d)	
3',5'	7.43 br t (8.0)	128.49 (d)	
4'	7.55 br tt (1.3, 7.5)	133.33 (d)	
7'	-	166.41 (s)	

* The ^1H-^{13}C connectivities and ^{13}C multiplicities were deduced according to HMQC and DEPT experiments.

[a] Assignments may be interchanged.

2.1.11 β-Sitosterol (95)

Stigmast-5-en-3-ol or 24-ethylcholest-5-en-3-ol is known as β-sitosterol. It is widely distributed in plants and is considered as the most common sterol of higher plants (93).

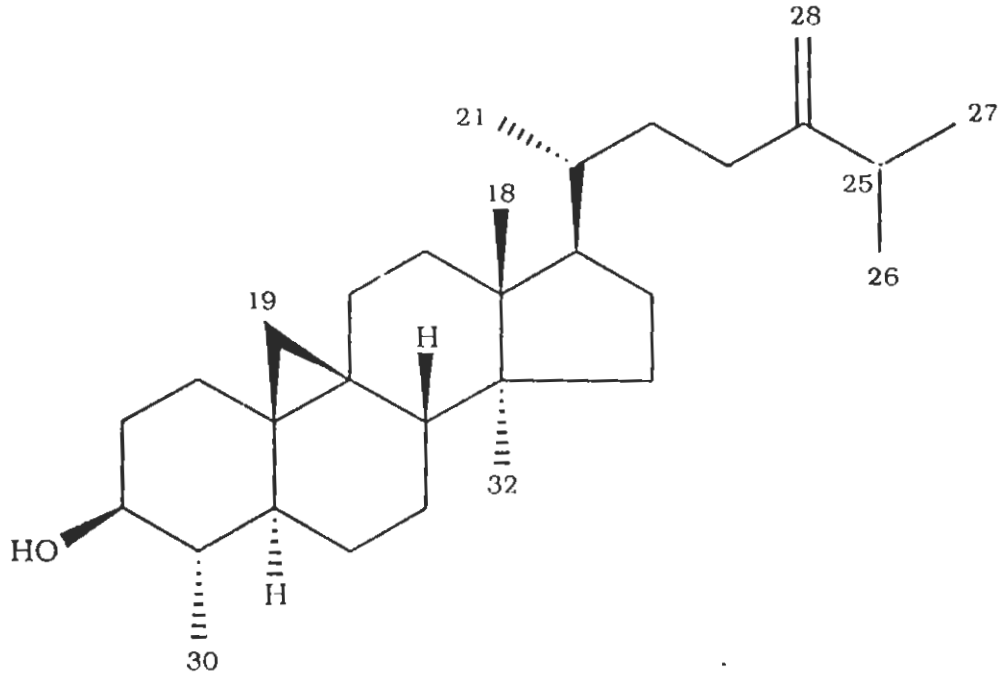


(95)

β-Sitosterol was purified from the 6th fraction (see experimental section) collected from the initial column chromatographic step and crystalysed from methanol. It was identified by comparison of EIMS and ^{1}H-NMR spectra with those data published in the literature (94-96).

2.1.12 Cycloeucalenol (96)

Cycloeucalenol, or 24-methylene-29-norcycloartan-3β-ol is a 24-methylene cycloartane triterpene and is widespread in . . plants of the genus *Euphorbia* (97). Cycloeucalenol, together with obtusifoliol, were isolated from the 7th fraction of the initial column chromatographic step (see experimental) and was purified by preparative TLC (silica gel F_{254}) using chloroform-methanol (60 ml : 6 drop).

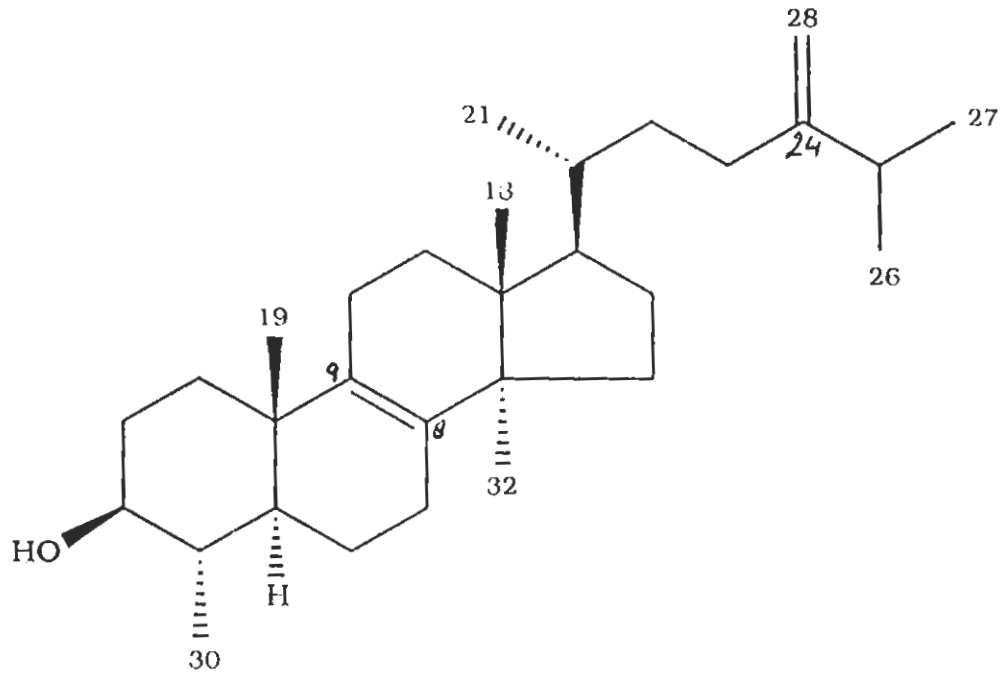


(96)

The identification of the compound was confirmed by comparison of the EIMS, ^{1}H and ^{13}C-NMR spectral data with those published in the literature (97, 98).

2.1.13 Obtusifoliol (97)

Obtusifoliol, or 4,14-dimethylergosta-8,24(28)-dien-3β-ol, is found in several different families of plants (42, 99-101).

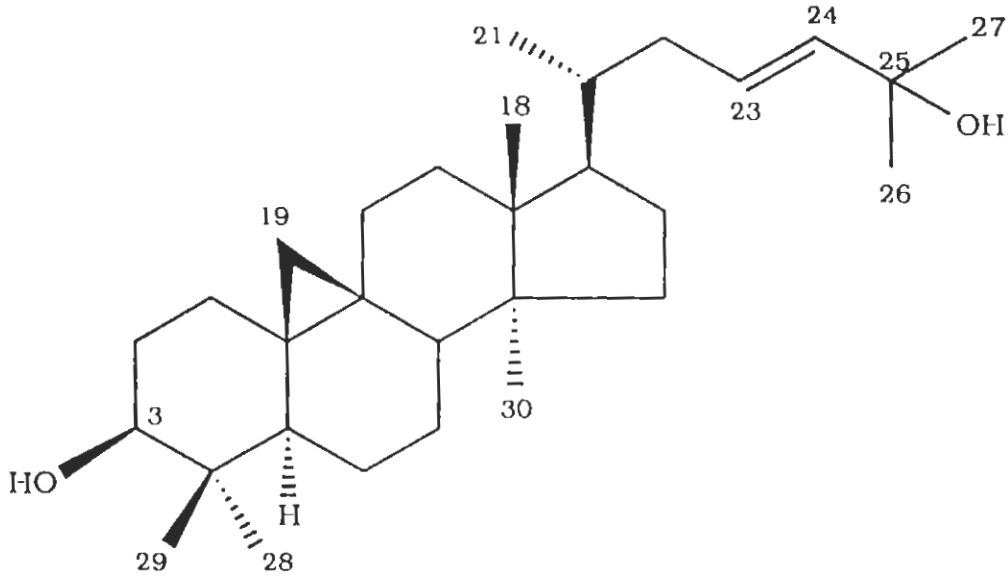


(97)

This compound was found as a major component with **96**, and was identified by comparison of its ^{1}H and ^{13}C-NMR data with those published in the literature (42, 99-101). In the ^{13}C-NMR and ^{1}H-NMR spectra of **97** two signals at δ 134.7 (C-9) and 133.7 (C-8) and also a singlet in the ^{1}H-NMR spectrum at δ 0.71 (H-18), were characteristic for distinguishing this compound from **96**.

2.1.14 Cycloart-23-en -3,25-diol (98)

Cycloart-23-en -3,25-diol (98) was isolated from the non-polar fraction collected from the initial column chromatographic step and purified by preparative TLC on precoated silica gel, using chloroform : methanol (60 ml : 6 drop). 98 showed significant cytotoxic activity against Ehrlich ascites tumor cells in culture (7).

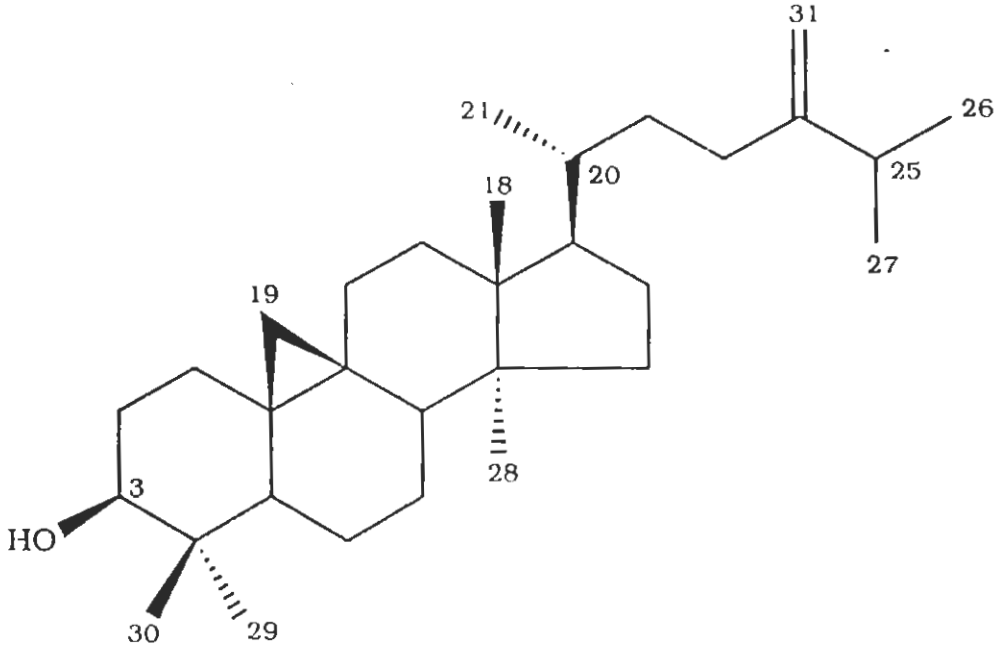


(98)

The identification was confirmed by comparison of the ^{1}H-NMR and mass spectral data with the literature (101, 102). Characteristic resonances were observed at δ 0.31 (d, J=4.1 Hz, H-19) and 0.53 (d, J=4.0 Hz, H-19'), together with a doublet of doublets at δ 3.26 (dd, J=4.3, 7.0 Hz, H-3) and a broad singlet at δ 5.58 (H-23, H-24).

2.1.15 24-Methylenecycloartan-3β-ol (99)

24-Methylenecycloartan-3β-ol is a constituent of rice bran oil and is found in some plants of the genus *Euphorbia* (100, 101). 99 is reported to be cytotoxic against P-388 cell system (*in vitro*) and antiproteolytic (103). It was isolated from the non-polar fraction by preparative TLC (silica gel F_{254}) using chloroform as mobile phase.



(99)

The identification of this compound was established using ^{1}H-NMR and mass spectral data and comparison with literature data (38, 101, 104). The characteristic peaks in ^{1}H-NMR spectrum were, a doublet of doublets at δ 3.27 (*J*=4.5, 11.3 Hz) and AB doublets at δ 0.32 (d, *J*=4.1 Hz) and 0. 54 (d, *J*=4.0 Hz), which indicated a cycloartane skeleton. Also two broad singlets at δ 4.66 and 4.70 an exocyclic methylene group.

2.2 Part B: Phytochemical Investigation of *Euphorbia teheranica* Boiss.

2.2.1 Introduction

Euphorbia teheranica Boiss. (Euphorbiaceae) is a plant which grows wild in Tehran and semidesertic areas of the central parts of Iran (5). This section describes the isolation and identification of three novel diterpene esters: tehranone A (3,5,10,14,15-*O*-pentaacetyl-8-*O*-2'-(methylbutanoyl)-cyclomyrsinol) (**100**), tehranone B (5,10,14,15-*O*-tetraacetyl-3-*O*-nicotinoyl-8-*O*-2'-(methylbutanoyl)-cyclomyrsinol) (**101**) and tehranol ester (3,5,7,15,17-*O*-pentaacetyl-14-*O*-nicotinoyl-17-hydroxymyrsinol) (**102**), together with methyl gallate (**105**), betulin (**106**), erythrodiol (**107**), oleanolic acid (**108**) and β-sitosterol glycoside (**109**) from *E. teheranica*.

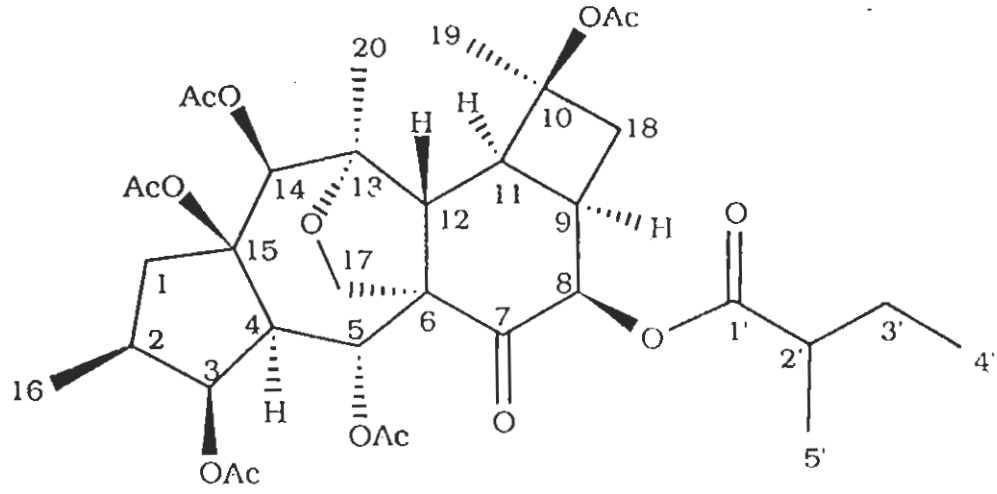
To the best of our knowledge, except a report on mild skin-irritant effects and tumor-promoting activity of this plant, there are no other studies. These activities are attributable to the presence of the short-chain esters of ingenol diterpenoids (105). The present study didn't show this type of diterpenoids in *E. teheranica*.

Diterpenoids containing the same cyclomyrsinol skeleton in literature (105) as tehranone, have already been reported from *E. prolifera*, and their structures were confirmed by X-ray crystallography (106). An ester withasimilar structure was isolated from *E. seguieriana* (65). The lithium aluminum hydride reduction of myrsinol, produced the 14β-hydroxy derivative as the major product, with the same configuration at

C-14 as tehranol ester (64). Recently, five esters with similar structures to tehranol ester but with the opposite configuration at C-14, together with a diterpene ester with the same carbon skeleton of tehranone, were identified in *E. seguieriana* (107).

2.2.2 Tehranone A (100)

Tehranone A (**100**) was obtained as a white powder from methanol. The compound was purified by preparative TLC using chloroform-acetone (9:1).



(**100**)

2.2.2.1 Structure Elucidation of tehranone A (100)

Compound **100** was assigned the molecular formula $C_{35}H_{48}O_{14}$ on the basis of CIMS *m/z* 691, $[M-1]^+$, and HREIMS *m/z* 633.2889 (calcd. for $C_{33}H_{45}O_{12}$, 633.2911) $[M+1-HOAc]^+$. The EIMS and CIMS mass spectra showed several losses of 60 mass units indicating acetate groups leaving the molecule as acetic acid, and also a peak at *m/z* 85 indicating the presence of a C_4H_9CO moiety in the molecule. The base peak at *m/z* 57 showed the butyl (C_4H_9) of the ester moiety. The IR spectrum established the presence of carbonyl groups of the ester moieties from a prominent absorption at 1740 cm^{-1}.

The ^{1}H-NMR spectrum (in $CDCl_3$, 500 MHz, Table 2.11) showed signals due to four oxymethine groups at δ 5.86 (dd, *J*=1.6, 11.0 Hz, H-5), 5.40 (t, *J*=4.3 Hz, H-3), 5.25 (d, *J*=6.3 Hz, H-8) and 5.02 (s, H-14), five singlets for acetates at δ 1.90, 1.95, 2.07, 2.08 and 2.18, signals for two methyls at δ 0.88 (t, *J*=7.5 Hz, H-4') and 1.29 (d, *J*=6.9 Hz, H-5') of a 2-methylbutanoate ester group, and three methyls at δ 0.85 (d, *J*=6.8 Hz, H-16), 1.18 (s, H-20) and 1.62 (s, H-19). Informative signals at δ 4.21 (d, *J*=9.7 Hz, H-17') and 3.58 (dd, *J*=1.6, 9.7 Hz, H-17) indicated the presence of the tetrahydrofuran ring of a myrsinane-type skeleton, which may be recognized from its long-range coupling constant of H-17 with H-5 (*J*=1.6 Hz) and the relatively small geminal J value (9.7 Hz) (64, 65). The other indicative ^{1}H-NMR signals for the determination of a myrsinane-type skeleton were the resonances at δ 4.03 (d, *J*=12.4 Hz, H-12) and a doublet of doublets at 2.90 (*J*=3.9, 11.0 Hz, H-4). The presence of a cyclobutane ring was established by the observation of a signal at δ 2.73 (dddd, J=6.9, 9.2, 9.2, 9.4 Hz, H-9) and the signals at 2.5 (m, H-18), and 2.40 (m, H-11) which were confirmed by a ^{1}H-^{1}H COSY 45° experiment (Fig. 2.9).

In the ^{13}C-NMR spectra (BB and DEPT, 75.4 MHz, Table-2.11), 35 signals were observed, in which ten were CH_3, four CH_2, ten CH, and eleven quaternary carbon atoms. The characterstic peaks for a cyclobutane ring were observed at δ 30.1 (d, C-9), 77.6 (s, C-10), 41.3 (d, C-11), and 35.1 (t, C-18). Also, the carbonyl group at δ 204.4 (s, C-7), and a doublet at 81.9 (C-14) were identified as specific resonances for the tchranone skeleton.

Using ^1H-^1H COSY 45° and HMQC spectra it was determined that the partial structures—CH_2—CH (CH_3)—CH (OR_1)CH—CH(OR_2)—and —CH(OR_3)—CH(CH_2)—CH—CH— were present, which were connected to each other through the quaternary carbons of C-6, C-7, C-10, C-13 and C-15.

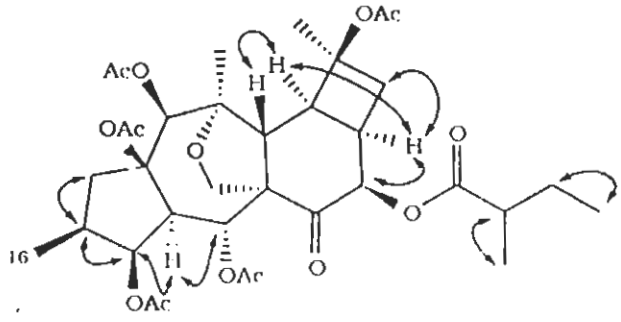
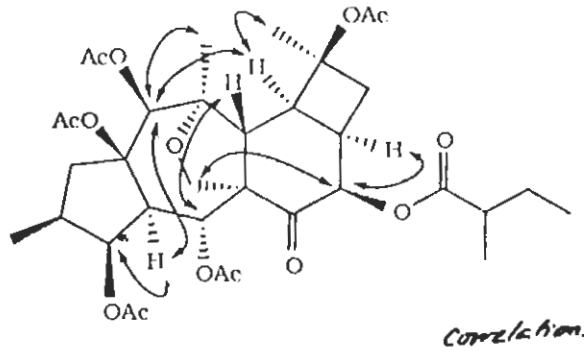


Figure 2.9: The ^1H-^1H COSY 45° correlations for tehranone A.

The relative positions of the ester groups were deduced from the cross peaks of the HMBC spectrum (Table-2.11). To confirm the ^1H-^1H-connectivities in cyclobutane ring and 2-methylbutanoate moiety, **100** was subjected to decoupling experiments in which irradiation of the signal at δ 2.60 (H-2') collapsed the doublet at δ 1.29 (Me-5') to a singlet, and irradiation of H-11 at δ 2.40 changed the H-12 signal to a doublet with a small J value. Irradiation of H-18 was not informative, however, the relative stereochemistry of **100** was determined according to NOESY spectrum (Fig. 2.10) and also comparison of the ^1H-NMR spectral data of the related compounds based on the myrsinane-type skeleton (64, 65, 105, 106).



correlations

Figure 2.10: The ^{1}H-^{1}H NOESY spectral for tehranone A.

Table-2.11: The spectral data of tehranone A (**100**)*.

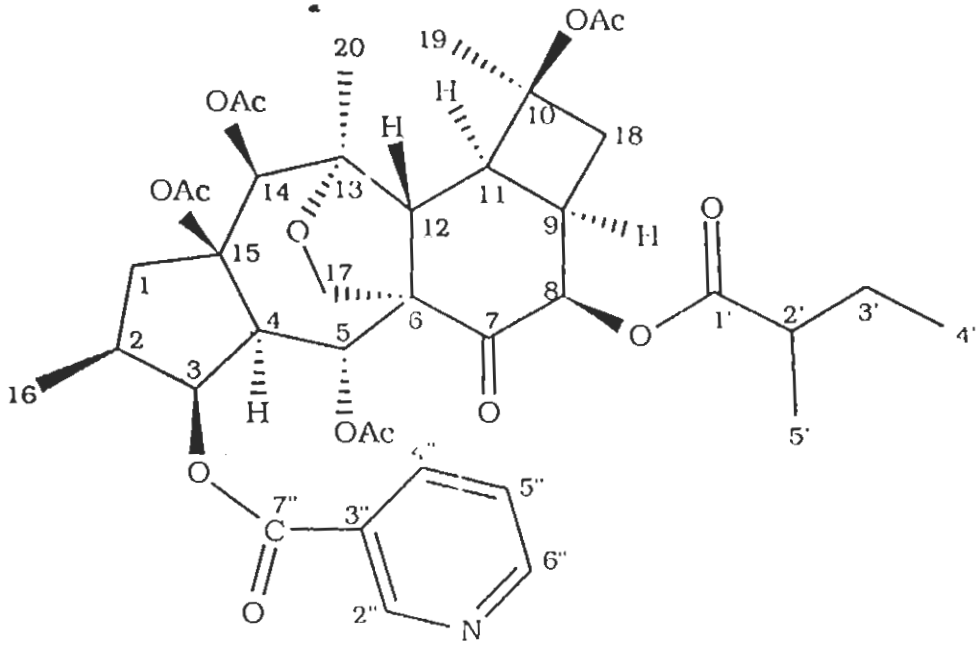
Position	^1H	^{13}C	HMBC Correlations
1α	2.85 dd (9.8, 16.0)	43.1 (t)	C-2, C-15, C-16
1β	2.49 dd (9.7, 16.0)		
2	2.3 m	35.7 (d)	
3	5.40 t (4.3)	77.6 (d)	$OCOCH_3$, C-1, C-15
4	2.90 dd (3.9, 11.0)	51.5 (d)	C-3, C-5
5	5.86 dd (1.6, 11.0)	68.7 (d)	$OCOCH_3$, C-4, C-6, C-17
6	-	62.2 (s)	
7	-	204.4 (s)	
8	5.25 d (6.9)	71.2 (d)	C-1', C-7, C-11, C-12
9	2.73 dddd (6.9, 9.2, 9.2, 9.4)	30.1 (d)	C-11, C-12
10	-	77.6 (s)	
11	2.40 m	41.3 (d)[a]	
12	4.03 d (12.4)	41.9 (d)[a]	C-5, C-6, C-10, C-11, C-13, C-17
13	-	89.3 (s)	
14	5.02 s	81.9 (d)	$OCOCH_3$, C-12, C-13, C-15
15	-	90.5 (s)	
16	0.85 d (6.8)	14.2 (q)	C-1, C-2, C-3
17	3.58 dd (1.6, 9.7)	67.2 (t)	C-6, C-12, C-13
17'	4.21 d (9.7)		
18	2.50 m	35.1 (t)	
18'	2.50 m		
19	1.62 s	24.5 (q)	C-10, C-11, C-18
20	1.18 s	23.3 (q)	C-12, C-13, C-14
2-Methyl-butanoyl			
1'	-	174.5 (s)	
2'	2.60 q (6.9)	41.0 (d)	
3'	1.70 m	27.0 (t)	
4'	0.88 t (7.5)	11.1 (q)	
5'	1.29 d (6.9)	15.9 (q)	C-1', C-3', C-4'
$OCOCH_3$[b]			
	1.90 s	20.6 (q)	
	1.95 s	20.8 (q)	
	2.07 s	21.3 (q)	
	2.08 s	21.6 (q)	
	2.18 s	23.4 (q)	
$OCOCH_3$			
	-	170.4 (s)	
	-	170.1 (s)	
	-	169.6 (s)	
	-	168.8 (s)	
	-	168.0 (s)	

* The ^1H -^{13}C connectivities and ^{13}C multiplicities were deduced according to HMQC and DEPT experiments.

[a,b] The assignments may be interchanged.

2.2.3 Tehranone B (101)

Tehranone B (**101**) was purified by preparative TLC using chloroform-acetone (9:1) as a white powder.



(101)

2.2.3.1 Structure Elucidation of tehranone B (101)

isolate exhibited a [M]$^+$ peak at m/z 755.3092 in the HREIMS, indicating a molecular formula of $C_{39}H_{49}O_{14}N$. In the EIMS, the ions at m/z 85 $[C_4H_9CO]^+$ and 57 $[C_4H_9]^+$, together with a m/z 106 $[C_6H_4ON]^+$ and 124 $[C_6H_5O_2N+1]^+$ established the presence of $C_4H_9CO_2R$ and nicotinoate ester units in the molecule. The IR spectrum gave signals for unsaturation (1580 and 740 cm^{-1}), besides signals for carbonyl absorption at 1740 cm^{-1}.

The ^{1}H and ^{13}C-NMR spectra of **101** (in $CDCl_3$, 500 MHz, Table 2.12) were similar to those recorded for **100**, except the signals at δ 9.09 (br s, H-2"), 8.79 (br s, H-6"), 8.26 (br d, J=8 Hz, H-4") and 7.45 (br s, H-5"), due to a nicotinoate moiety. The positions of the ester groups were determined by HMBC cross peaks between H-8 and carbonyl carbon of the 2-methylbutanoate unit at δ 174.5, and between H-14 and the carbonyl carbon of an acetate. The position of the other acetate groups and the nicotinoate ester functionalities were deduced from the downfield shift of H-3 (0.32 ppm) in comparison to **100**, which was the result of substitution of the nicotinoate at C-3 in **101** and upfield shift of H-16 (0.23 ppm), that can be considered as a consequence of the existence of Me-16 in the shielding anisotropic field of the nicotinoate aromatic ring. To confirm the relative configuration of **101**, it was subjected to a NOE difference experiment. Irradiation of the signal at δ 5.11 (H-14) gave a significant enhancement of the Me-19 (2%) and Me-20 signals, while irradiation of H-8 enhanced the Me-19 (1.6%) signal. Irradiation of H-4 enhanced the signals of H-3 (7.5%), H-5 (6.7%) and H-4" (7.1%). Finally, H-5 showed a correlation with H-12, and a correlation between H-16 and H-4" (12.7%) confirmed the location of nicotinate moiety.

Table-2.12 : The spectral data of tehranone B (**101**)*.

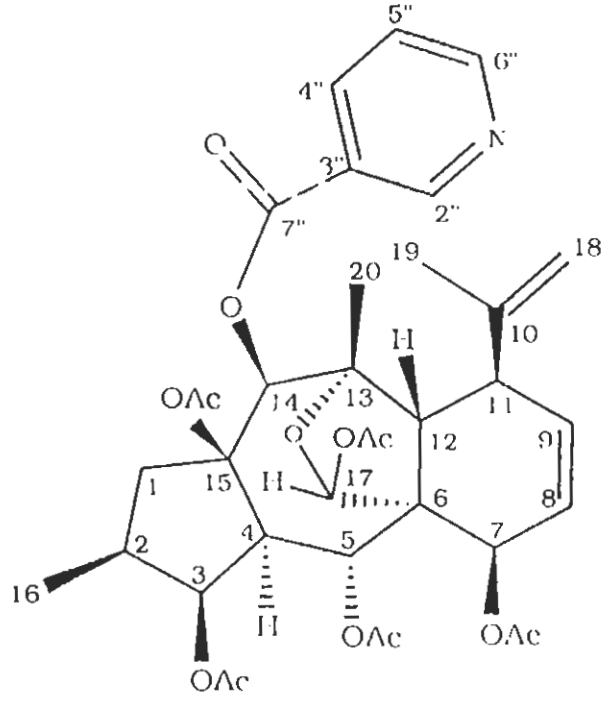
Position	^1H	^{13}C	HMBC *Correlations*
1α	2.94 dd (11.2, 16.1)	43.6 (t)	
1β	2.4 m		
2	2.2 m	36.4 (d)	
3	5.72 t (3.8)	77.5 (d)	C-2, C-5, C-15
4	3.10 dd (3.6, 11.1)	51.4(d)	C-5
5	5.86 dd (1.1, 11.1)	68.4 (d)	
6	-	62.0 (s)	
7	-	204.2 (s)	
8	5.19 d (7)	71.0 (d)	C-7, C-11, C-1'
9	2.70 m	30.0 (d)	
10	-	78.0 (s)	
11	2.40 m	41.4(t)[a]	
12	4.09 d (12.3)	41.9 (d)[a]	C-5, C-11,
13	-	89.5 (s)	
14	5.11 s	81.8 (d)	$OCOCH_3$, C-12, C-13, C-15
15	-	90.1 (s)	
16	0.62 d (6.8)	14.2 (q)	
17	3.60 dd (1.5, 9.8)	67.3 (t)	C-5, C-12, C-13
17'	4.25 d (9.8)		
18	2.50 m	35.1 (t)	
18'	2.50 m		
19	1.63 s	24.4 (q)	C-10, C-11, C-18
20	1.21s	22.4 (q)	C-12, C-13, C-14
2-Methyl-butanoyl			
1'	-	174.5 (s)	
2'	2.70 m	40.9(d)	
3'	1.60 m	26.9 (t)	
4'	0.78 t (7.4)	11.0 (q)	
5'	0.93 d (6.7)	15.5 (q)	
$OCOCH_3$[b]			
	1.93 s	20.8 (q)	
	2.10 s	21.3 (q)	
	2.12 s	21.7 (q)	
	2.31 s	23.9 (q)	
$OCOCH_3$			
	-	170.6 (s)	
	-	169.7 (s)	
	-	168.8 (s)	
	-	168.2 (s)	
Nicotinoyl			
2"	9.09 br s	-	
4"	9.26 br d (8)	-	
5"	7.45 br s	-	
6"	8.79 br s	-	

* The ^1H-^{13}C connectivities and ^{13}C multiplicities were deduced according to HMQC and DEPT experiments.

[a,b] The assignments may be interchanged.

2.2.4 Tehranol ester (102)

Tehranol ester (102) was obtained as a white powder from methanol. The compound was purified by preparative TLC (silica gel F_{254}) using chloroform-acetone (9:1).



(102)

2.2.4.1 Structure Elucidation of tehranol ester (102)

was assigned the molecular formula $C_{36}H_{43}O_{13}N$ on the basis of HREIMS (*m/z* 697.2758). Its IR showed strong absorptions for ester carbonyls (1730 cm^{-1}) and unsaturation (1590 cm^{-1}). In the EIMS, the peaks at *m/z* 637 $[M\text{-}HOAc]^+$, 517 $[M\text{-}3 \times HOAc]^+$, and the prominent peaks at *m/z* 106 $[C_6H_4ON]^+$ and 124 $[C_6H_5O_2N+1]^+$, suggested the presence of acetate and nicotinoate esters in the molecule.

The ^{1}H-NMR spectrum of **102** (Table 2.13) was similar to the spectrum of compounds with the myrsinol skeleton, except for a singlet at δ 5.21 (s, H-14), which resulted from the reduction and esterification of the carbonyl group at C-14. The other difference was caused by hydroxylation and acetylation at C-17, that changed the typical AB doublets of H-17 and H-17' to a singlet at δ 6.36. The spectrum also showed signals for five acetate groups and one nicotinate unit at δ 1.98, 1.99, 2.00, 2.01, and 2.07, and δ 9.03 (s, H-2"), 8.80 (br s, H-6"), 8.40 (br s, H-4") and 7.45 (br s, H-5"), respectively. The correct multiplicities of the nicotinoate moiety were only obtained when the solvent was changed to C_6D_6 (Table-2.14).

In the ^{13}C-NMR spectra, (in $CDCl_3$, 75.4 MHz, Table 2.13) signals at δ 97.8 and 81.6 were observed for C-17 and C-14, respectively. For determination of the position of the esters, **102** was subjected to an HMBC experiment (in $CHCl_3$), but some of the correlations (H-3, H-7/$OCOCH_3$) could not be deduced. Then the solvent was changed to benzene to help in determining the exact position of the esters (Table 2.14). A correlation between H-14 and C-7" in both HMBC experiments established the position of the nicotinoate ester at C-14. The relative configuration of compound was assigned by ^{1}H-NMR coupling constants and NOESY spectra (Fig. 2.11). The configuration at C-17 was confirmed using NOE difference spectroscopy experiments (Fig. 2.12). Irradiation of both H-4 and H-7 enhanced the H-17 signal at δ 6.36. A significant enhancement of H-3 and H-14 on irradiation of H-4, confirmed the configuration of H-14 as α. On the other hand, the lack of the W-type long range coupling between H-5 and H-17, can be another proof for the suggested structure.

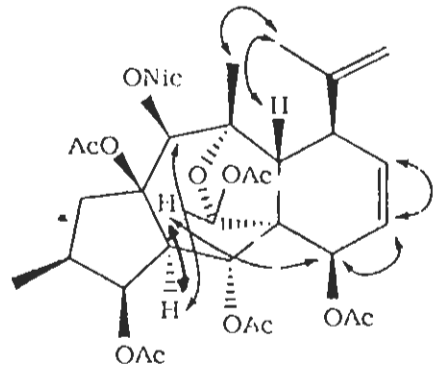


Figure 2.11: ^{1}H-^{1}H NOESY spectral data of **102**.

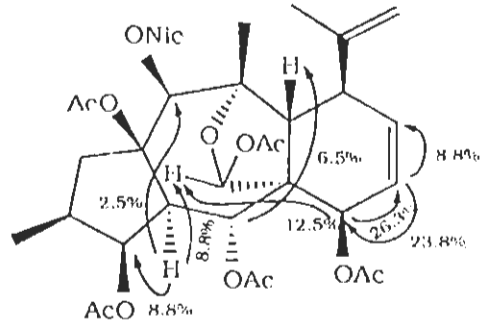
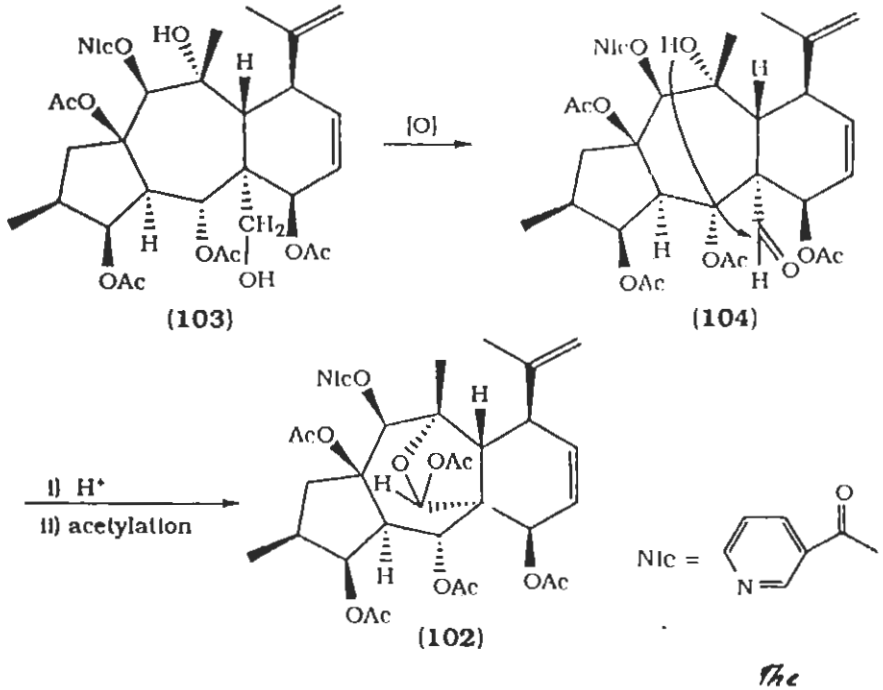


Figure 2.12: The NOE difference spectral data (irradiated ^{1}H $\xrightarrow{\%}$ enhanced ^{1}H) of **102**.

2.2.4.2 Biogenesis of tehranol ester (102) from an decipinone Precursor (103)

The hemiacetal ring in **102** and related compounds prompted us to propose a new pathway for the biosynthesis of this category of diterpenoids. Beginning with an decipinone precursor, (**103**), which was oxidized at C-17 to give an aldehyde **104** . The hemiacetal formation and acetylation of C-17 free hydroxyl group in the hemiacetal resulted in the tehranol ester formation (Scheme 2.5).



Scheme-2.5: Proposed biogenesis of the tehranol ester skeleton (**102**) from a decipinone skeleton (**103**).

Table-2.13: The spectral data of tehranol ester (**102**) (in $CDCl_3$)*.

Position	^1H	^{13}C	HMBC Correlations
1α	2.66 m	43.9 (t)	
1β	2.66 m		
2	2.2 m	36.9 (d)	
3	5.18 t (3.6)	76.4 (d)	
4	3.05 dd (3.5, 11.1)	52.0 (d)	
5	6.04 d (11.1)	69.4 (d)	$OCOCH_3$, C-4,C-17
6	-	56.6 (s)	
7	5.04 d (5.9)	63.3 (d)	
8	6.01 ddd (10.0, 6.0, 2.2)	123.2 (d)	
9	5.73 dd (4.2, 10.0)	133.6(d)	
10	-	147.4 (s)	
11	3.3 m	43.8 (d)	
12	3.3 m	37.2 (d)	
13	-	89.6 (s)	
14	5.21 s	81.6 (d)	C-7", C-13, C-15
15	-	91.0 (s)	
16	0.82 d (6.7)	14.2 (q)	C-1, C-2, C-3
17	6.36 s	97.8 (d)	$OCOCH_3$, C-12, C-13
18	4.92 br s	114.1 (t)	
18'	5.14 br s		
19	1.86 s	18.8 (q)	C-10, C-18
20	1.39s	24.9 (q)	C-13, C-14
$OCOCH_3$[a]			
	1.98 s	22.5 (q)	
	1.99 s	21.3 (q)	
	2.00 s	21.2 (q)	
	2.01 s	21.0 (q)	
	2.07 s	20.7 (q)	
$OCOCH_3$			
	-	170.7 (s)	
	-	170.0 (s)	
	-	169.5 (s)	
	-	168.9 (s)	
	-	168.5 (s)	
Nicotinoyl			
2"	9.02 br s	151.1 (d)	
3"	-	126.1 (s)	
4"	8.40 br s	138.2 (d)	
5"	7.54 br s	123.6 (d)	
6"	8.80 br s	153.7 (d)	
7"	-	163.8 (s)	

* The ^1H-^{13}C connectivities and ^{13}C multiplicities were deduced according to HMQC and DEPT experiments.

[a] The assignments may be interchanged.

Table-2.14: The spectral data of tehranol ester (102) (in C_6D_6)*.

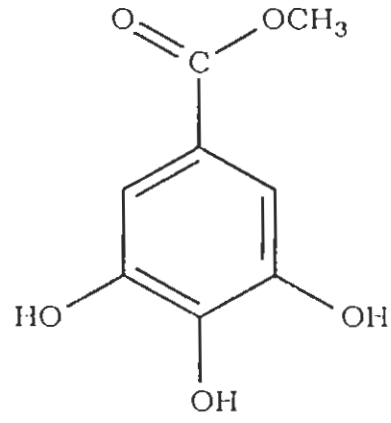
Position	^1H	^{13}C	HMEC Correlations
1α	2.79 dd (9.1, 15.9)	47.1 (t)	
1β	2.95 dd (11.0, 15.9)		
2	1.5 m	36.9 (d)	
3	5.05 t (3.7)	76.4 (d)	$OCOCH_3$, C-1, C-15
4	2.92 dd (4.0, 11.2)	52.1 (d)	
5	6.22 d (11.2)	69.8 (d)	
6	-	57.2 (s)	
7	5.41 d (5.9)	63.6 (d)	$OCOCH_3$
8	6.14 ddd (2.2, 5.9, 9.9)	123.9 (d)	
9	5.58 dd (4.3, 9.9)	133.9 (d)	
10	-	147.6 (s)	
11	3.34 m	44.0 (d)	
12	3.48 d (5.3)	37.8 (d)	
13	-	89.9 (s)	
14	5.45 s	81.2 (d)	C-7", C-12, C-13, C-15
15	-	91.6 (s)	
16	0.72 d (6.7)	14.1 (q)	
17	6.78 s	98.2 (d)	$OCOCH_3$, C-12, C-13
18	4.9 br s	113.6 (t)	
18'	5.1 br s	-	
19	1.57 s	19.0 (q)	
20	1.49 s	25.0 (q)	
$OCOCH_3$[a]			
	1.84 s	22.1 (q)	
	1.84 s	21.1 (q)	
	1.87 s	20.8 (q)	
	1.91 s	20.7 (q)	
	1.93 s	20.5 (q)	
$OCOCH_3$			
	-	170.1 (s)	
	-	169.4 (s)	
	-	169.2 (s)	
	-	168.9 (s)	
	-	167.7 (s)	
Nicotinoyl			
2"	9.48 br d (2.2)	151.5 (d)	
3"	-	126.3 (s)	
4"	8.26 dt (1.8, 8.0)	137.3 (d)	
5"	6.67 ddt (0.9, 4.8, 7.9)	123.2 (d)	
6"	8.51 dd (1.8, 4.8)	153.8 (d)	
7"	-	164.7 (s)	

* The ^1H-^{13}C connectivities and ^{13}C multiplicities were deduced according to HMQC and DEPT experiments.

[a] The assignments may be interchanged.

2.2.5 Methyl gallate (105)

Methyl gallate is the methyl ester of gallic acid, which is widespread in many tannins and can be a product of their degradation. This compound has shown different biological activities, such as antitumour, antibacterial and enzyme inhibitory properties (108, 109). It is also used in photographic developing (110).

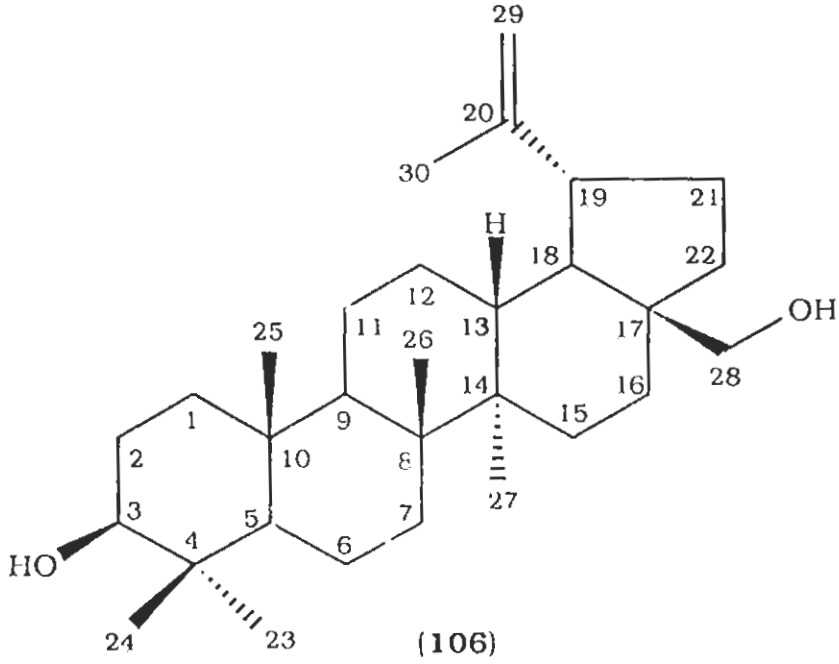


(105)

Compound **105** was separated from a 50% hexane-acetone fraction obtained from the initial column chromatographic step and purified by column chromatography (silica gel, 230-400 mesh) using chloroform-acetone (4:1). It was obtained as a white precipitate (Me_2CO), m.p. 196-198°C (literature m.p. 202°C) and exhibited spectroscopic data which were closely compatible to literature values (111, 112).

2.2.6 Betulin (106)

Betulin or 20 (29)-lupene-3β, 28-diol was purified from a mixture with **107** using column chromatography on silica gel impregnated with $AgNO_3$ as a white powder (see experimental section).



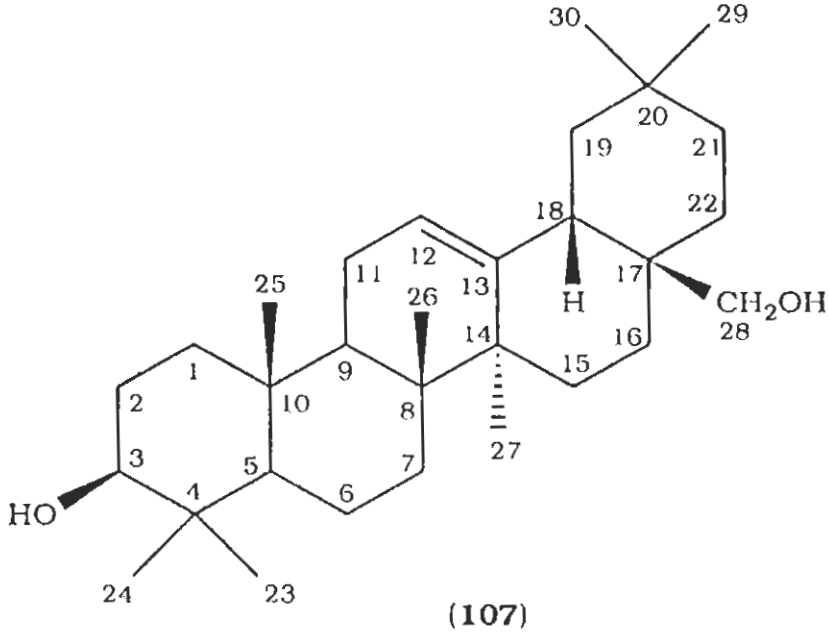
(106)

In its ^{1}H-NMR spectrum the characteristic peaks at δ 3.17 (dd, *J*=5.1, 10.9 Hz, H-3), 3.31 (dd, *J*=0.3, 11.0 Hz, H-28), 3.78 (dd, *J*=1.9, 10.9 Hz, H-28'), 4.56 (m, H-29), 4.66 (br d, H-29') and six tertiary methyl signals at δ 0.75 , 0.81, 0.96, 0.97, 1.01 and 1.67 represented a lupene diol skeleton in which one of the methyl groups was oxidized to an alcohol.

The structure of this compound was determined by comparison of the spectral data with those reported for an authentic sample in the literature (112-116).

2.2.7 Erythrodiol (107)

Erythrodiol, or 12-oleanene-3,28-diol (**107**), is a constituent of many plants, and is also widely distributed as an aglycone of different saponins. Erythrodiol was separated from a mixture with betulin by chromatography on silica gel impregnated with $AgNO_3$ (see the experimental section).

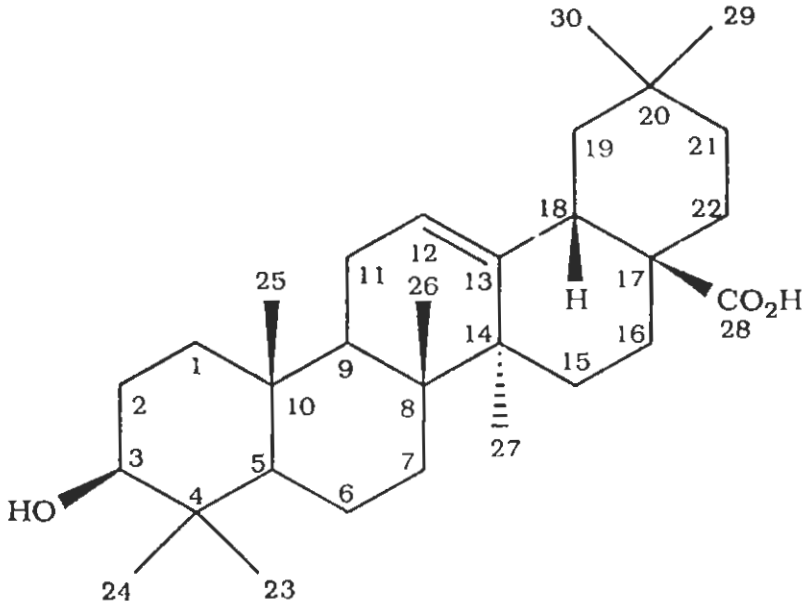


(107)

The spectral data, ^1H-, ^{13}C-NMR and EIMS, were in a good agreement with those published earlier, so identification of the compound was confirmed by comparison of the data with literature values (113, 118-120).

2.2.8 Oleanolic acid (108)

Oleanolic acid occurs as a glycoside in mistletoe, cloves, sugar beet, olive leaves and many other plants. It is also a widely distributed aglycone. This compound was purified by repeated column chromatography using chloroform-acetone.

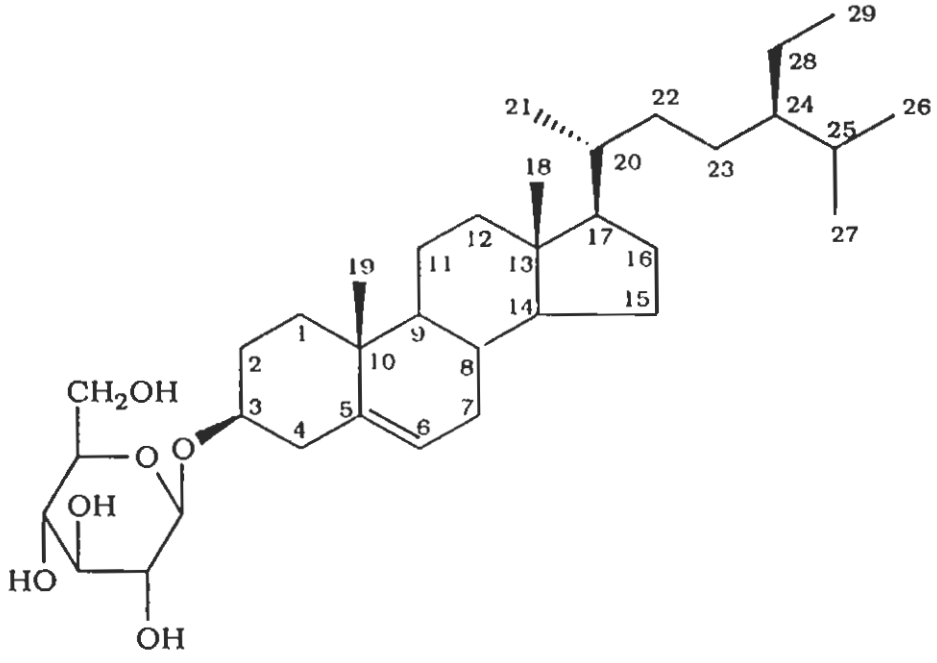


(108)

The identification of the compound was performed by comparison of the EIMS and ^{1}H-NMR spectral data with literature value[5] (120).

2.2.9 β-Sitosterol glycoside (109)

β-Sitosterol glycoside (**109**) is found in many different species of plants. This compound was separated from the polar fractions of the initial column chromatography.



(**109**)

Identification of the compound was accomplished by comparison of the spectral data with the literature (121, 122).

2.3 Part C: Constituents of the Essential oil of *Zataria multiflora* Boiss.

2.3.1 Introduction

Zataria multiflora Boiss, belonging to the family Lamiaceae is a small fragrant shrub which grows wild in the south of Iran, Pakistan and East of Afghanistan. The flowering period of this plant is between May and October. Its generic name is derived from the Arabic word Za'atar, meaning thyme, and although it is reported that this plant has a thyme like fragrance and there is slight resemblance to some Mediterranean thymus and origanum, However the affinities of the genus are uncertain and it is reported as an isolated genus (123).

Some medicinal properties have been attributed to this plant such as stimulant, diaphoretic and diuretic which may be due to its essential oil. Its infusion is valued as an aromatic stimulant, and as a good cure for stomach ache. Some biological activities such as antiseptic, analgesic and carminative properties are also attributed to this plant (124-126).

Recently, in the pharmacology Department of our Institute, the volatile oil of this plant was investigated for its spasmolytic activity and the results showed that it inhibited the spontaneous activity of the gut tissues from the rat and rabbit. Rat duodenum and rabbit jejunum showed 96.6% ± 1.9 (13 µ g/ml) and 100% ± 0 0 (55 µg/ml) inhibition of the spontaneous activity, respectively. The acetylcholine induced contractions were also reduced in rat gut tissues. So the above

observation suggested that the oil of *Z. multiflora* possesses spasmolytic activity.

The chemical constituents of *Z. multiflora* were previously investigated by Gupta *et al.* using GC. The essential oil (0.6% yield) was reported to consist of 69% phenols, mainly carvacrol, and *p*-cymene as the major non-phenolic portion of this oil. Alkanes, including, *n*-nonacosane (C_{29}), *n*-hentriacontane (C_{31}), *n*-dotriacontane (C_{32}), *n*-tritriacontane (C_{33}) and *n*-pentatriacontane (C_{35}), together with steroids and triterpenes, β-sitosterol, betulin, and oleanolic acid, and the fatty acids behenic acid (C_{22}); lignoceric acid (C_{24}), cerotic acid (C_{26}) and montanic acid (C_{28}), were identified in the light petroleum ether, benzene, ethanol and methanol extracts of this plant (124).

The chemical constituents of the essential oils of *Z. multiflora* from India and Pakistan were explored by Farooq (127) and Malik (125), respectively, using chemical tests and GC analysis. Recently, Shafiee and Javid studied the composition of the essential oil of an Iranian specimen using GC, MS, and in case of some of the constituents ^{1}H-NMR spectroscopy methods (126).

2.3.2 Qualitative and Quantitative Analysis of the Essential Oil of *Zataria multiflora*

Hydrodistillation of the fresh aerial parts (560 g) of *Zataria multiflora* yielded 12.2 g of a light yellowish oil (2.2% w/w yield). The oil after preparation was subjected to gas chromatography (GC) and gas chromatography/ mass spectrometry (GC/MS) using non-polar and

polar capillary columns. The components of the oil were characterized by comparison of their retention indices and mass spectra with literature values (128-134) and their concentrations were determined using area normalization with a C-R6A integrator coupled to GC. The oil was then resolved into two fractions, using hexane (non polar) and ethyl acetate (polar). Each fraction after removing the solvent in vacuum, was subjected to ^{13}C-NMR spectroscopy techniques, including broad band (BB) and DEPT. Comparison of the ^{13}C-NMR spectral data of the oil components with those reported for the pure compounds in the literature (74, 135) confirmed the identification by GC/MS.

In the case of thymol, thymol acetate, carvacrol and carvacrol acetate the identification was further confirmed by co-injection of the standard compounds using the same chromatographic conditions.

Table-2.15 shows the result of the qualitative and quantitative analysis of the essential oil of *Z. multiflora*. The oil consisted mainly of *p*-menthane monoterpenes including, carvacrol (62 4%), methyl carvacrol (6.5%), carvacrol acetate (4.4%), *p*-cymene (7.1%), γ-terpinene (5.6%), 1,8-cineole (0.5%), terpinolene (0.4%), carvone (0.1%), terpinone-4-ol (0.1%), α-terpineol (1.1%), thymol (2.0%) and thymol acetate (0.6%), which comprised 90.8% of the oil.

The other monoterpenes, including tricyclene (0.9%), α-thujene (2.0%), α-pinene (0.1%), camphene (0.4%), β-pinene (0.8%) and myrcene (0.2%), consisted of 4.4 % of the oil. Finally, the sesquiterpenes β-caryophyllene

(0.6%), aromadendrene (0.8%), α-himachalene (0.2%), valencene (0.9%), 9-aristolene-1-α-ol (1.6%) and guaiol (0.2%), were determined as minor fractions (4.3%) of the total oil.

The results reported for the essential oil of *Z. multiflora* by different authors are compared in Table-2.16. The first investigation on this plant from India was carried out by Farooq and Gupta in 1954, in which only two compounds, p-cymene and borneol were identified (127). In another report Gupta reported carvacrol as the major compound (124). The only paper on the Pakistani species of this plant in which the components were identified by gas chromatography, was by Malik et al (125). Recently, Shafie and Javidnia characterized the essential oil of this plant, collected from Iran using GC, GC/MS and in some cases, ^{1}H-NMR spectroscopy (126).

The results of the oil reported from India by Farooq were rather old, and Malik also used only GC data for the identification of the compounds. A comparison between our sample and the Iranian species showed that eugenol and methyl eugenol in Malik's sample may be mis-identified.

Both the Iranian sample and our sample consisted mostly of carvacrol, but the concentration of thymol in our sample was rather low. We didn't detect the non-terpenic compounds, 1-octen-3-ol and 3-octanone together with monoterpenes α-phellandrene, α-terpinene, limonene, linalool oxides, linalool, methyl thymol, bornyl acetate and the sesquiterpenes alloaromadendrene, α-humulene, spathulenol and widdrol, while 9-aristolen-1-α-ol, α-himachalene, guaiol, tricyclene,

1,8-cineol, carvone and carvacrol acetate were identified in this plant for the first time.

Table-2.15: Composition of the Essential oil of *Zataria multiflora* Boiss.

Compound	%	PI	Identification
tricyclene	0.9	919	GC/MS
α-thujene	2.0	927	GC, MS, ^{13}C-NMR
α-pinene	0.1	939	GC, MS
camphene	0.4	966	GC, MS
β-pinene	0.8	976	GC, MS
myrcene	0.2	993	GC, MS
p-cymene	7.1	1015	GC, MS, ^{13}C, ^{1}H-NMR
1,8-cineole	0.5	1020	GC, MS
γ-terpinene	5.6	1052	GC, MS, ^{13}C, ^{1}H-NMR
terpinolene	0.4	1073	GC, MS
terpinen-4-ol	0.1	1163	GC, MS
α-terpineol	1.1	1174	GC, MS
carvone	0.1	1208	GC, MS
methyl carvacrol	6.5	1232	GC, MS, ^{13}C-NMR
thymol†	2.0	-	GC, MS, Co-inj.
carvacrol	62.4	1325	GC, MS, ^{13}C, ^{1}H-NMR
thymol acetate	0.6	1345	GC, MS, Co-inj.
carvacrol acetate	4.4	1372	GC, MS, Co-inj.
β-caryophyllene	0.6	1420	GC, MS
aromadendrene	0.8	1441	GC, MS
α-himachalene††	0.2	1458	GC, MS
valencene	0.9	1493	GC
9-aristolen-1-α-ol††	1.6	1571	GC, MS
guaiol	0.2	1592	GC, MS

† The concentration was determined by a Supelcowax ™10 column.

†† Identification was mainly done on the basis of mass spectral data.

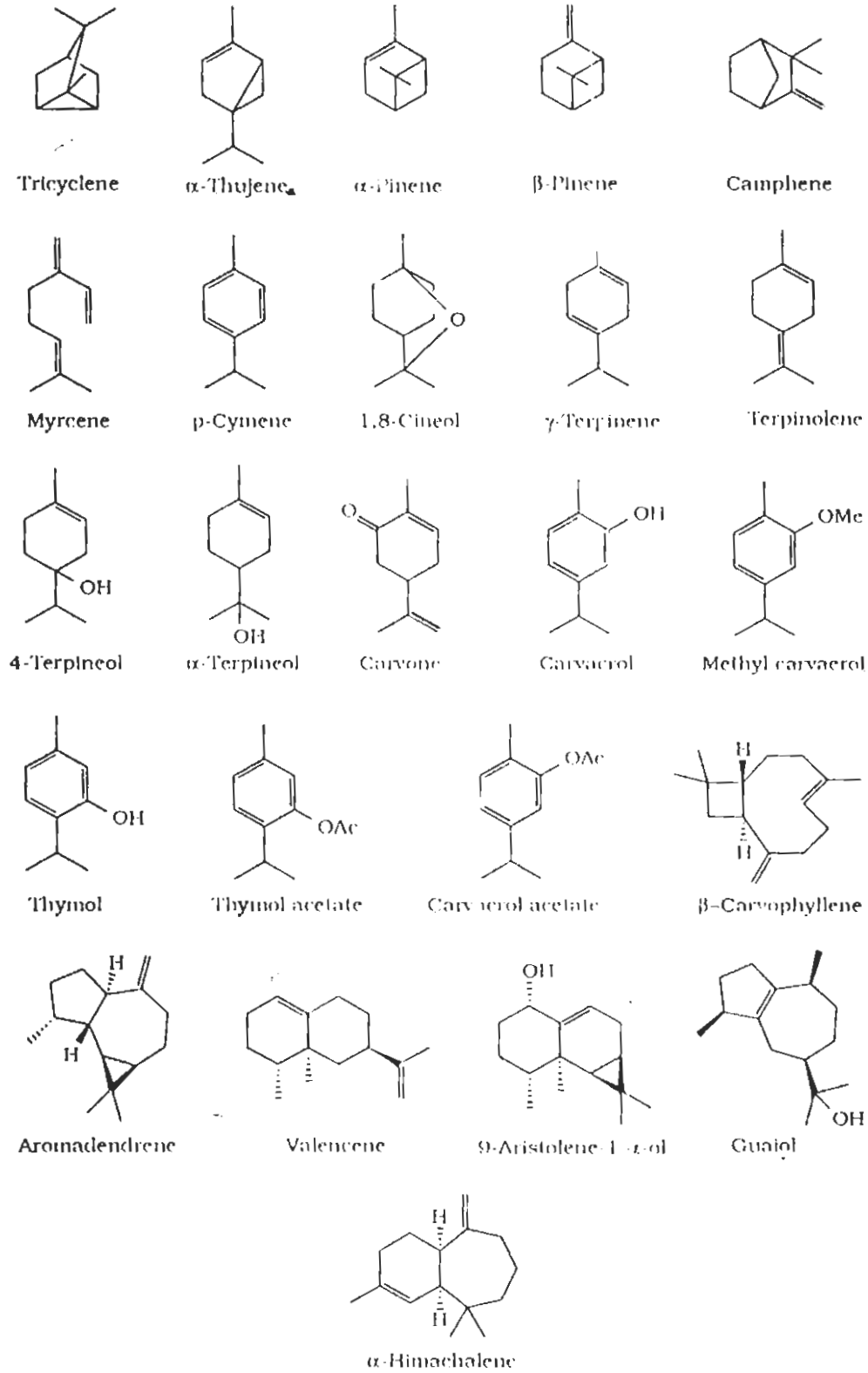


Figure-2.13: The structure of the chemical constituents of the essential oil of *Zataria multiflora*.

Table-2.16: The chemical constituents of the essential oil of *Z. multiflora* identified by different authors.

Constituent	Shafee (126) Dried sample	Shafee (126) Fresh sample	Farooq (127)	Malik (125)	Present study
tricyclene	-	-	-	-	0.9
α-thujene	0.10	0.97	-	-	2.0
α-pinene	0.34	2.28	-	-	0.1
camphene	0.01	0.08	-	-	0.4
1-octen-3-ol	0.02	0.19	-	-	-
3-octanone	0.05	0.64	-	-	-
β-pinene	0.09	0.40	-	-	0.8
myrcene	0.27	1.06	-	-	0.2
α-phellandrene	0.02	0.06	-	2.4	-
α-terpinene	0.33	1.35	-	-	-
p-cymene	1.90	13.5	17.0	7.1	7.1
limonene	0.21	0.46	-	-	-
β-phellandrene	0.18	0.43	-	-	-
γ-terpinene	0.76	3.95	-	0.35	5.6
linalool oxide trans (furanoid)	0.07	0.18	-	-	-
linalool oxide cis (furanoid)	0.06	0.17	-	-	-
linalool	1.96	5.20	-	-	-
4-terpineol	0.80	1.01	-	-	0.1
α-terpineol	0.54	0.67	-	-	1.1
thymol methyl ether	0.60	1.20	-	-	-
methyl carvacrol	0.95	1.20	-	-	6.5
thymol	25.18	48.4	-	15.5	2.0
bornyl acetate	0.01	0.04	-	-	-
carvacrol	61.29	12.6	-	57.4	62.4
carvyl acetate	0.02	0.01	-	-	-
β-caryophyllene	1.82	1.85	-	8.3	0.6
aromadendrene	0.23	0.20	-	-	0.8
alloaromadendrene	0.11	0.10	-	-	-
α-humulene	0.02	0.02	-	-	-
valencene	0.14	0.11	-	-	0.9
spathulenol	0.37	0.38	-	-	-
widdrol	0.64	0.38	-	-	-
borneol	-	-	3.0	1.7	
eugenol	-	-	-	1.7	-
methyl eugenol	-	-	-	1.5	-
1,8-cineole	-	-	-	-	0.5
terpinolene	-	-	-	-	0.4
carvone	-	-	-	-	0.1
thymol acetate	-	-	-	-	0.6
carvacrol acetate	-	-	-	-	4.4
guaiol	-	-	-	-	0.2
α-himachalene	-	-	-	-	0.2
9-aristolen-1α-ol	-	-	-	-	1.6

3. EXPERIMENTAL

3.1 General Experimental Procedures

The melting points were uncorrected and were measured by a Büchi apparatus 535. Optical rotations were measured on a JASCO DIP-360, polarimeter. The UV spectra were recorded on a Hitachi U-3200 instrument. The infrared (IR) spectra were recorded on a Shimadzu IR-460 and JASCO IR-302 instruments, using KBr or in chloroform. ^{1}H-NMR spectra were taken mostly in $CDCl_3$ at 500 MHz or 400 MHz on a Bruker AMX 500 Fourier Transform Spectrometer or on a Bruker AM-400 nuclear magnetic resonance spectrometer using TMS as an internal standard. The ^{13}C-NMR spectra were taken at 125 MHz or 75.4 MHz on a Bruker 500 and Bruker 300 in $CDCl_3$ and TMS as an internal standard, collecting between 2000 and 2500 pulses on samples between 10-40 mg. The solvents for chromatography were purified by fractional distillation. The reagents and silica gel were obtained from Merck and Fluka. Thin layer chromatography was carried out using precoated silica gel GF-254 preparative plates (20 x 20 cm) of Merck. Column chromatography was carried out using silica gel type-60 of E. Merck (70-230 mesh). Silica gel (230-400 mesh) was used for flash chromatography.

Detection of the chromatograms of TLC was carried out under ultraviolet light at 254 and 366 nm or by ceric sulfate solution in sulfuric acid followed by heating at 110°C. The purity of the samples was checked on TLC (Merck pre-coated sheets, silica gel 60 F_{254}).

X-Ray crystallography analysis was carried out on an Enraf Nonius CAD-4 diffractometer with Cu-Kα radiation. The structures were solved by direct methods, and expanded, using fourier techniques. The values for the mass attenuation coefficients are those of Creagh and Hubbell. All calculations for data reduction were performed using TEXSAN, crystallographic software package of Molecular Structure Corporation and the refinement was carried out with the aid of SHELX93.

3.2 Part A: Phytochemical Investigation of *Euphorbia decipiens*

3.2.1 Plant Material

Euphorbia decipiens Boiss. & Buhse grows wild in different parts of Iran at high altitudes (5). It was collected in July 1995 from the mountain Kandovan, north of Karaj, Tehran, Iran.

The plant was identified by Dr. Fereydoon Terme at "The Center for Plant Diseases and Pest Research," Eveen, Tehran, Iran. A voucher specimen was deposited at the herbarium of the Biology Department of Shahid Beheshti University Eveen, Tehran, Iran (Voucher No. 98112).

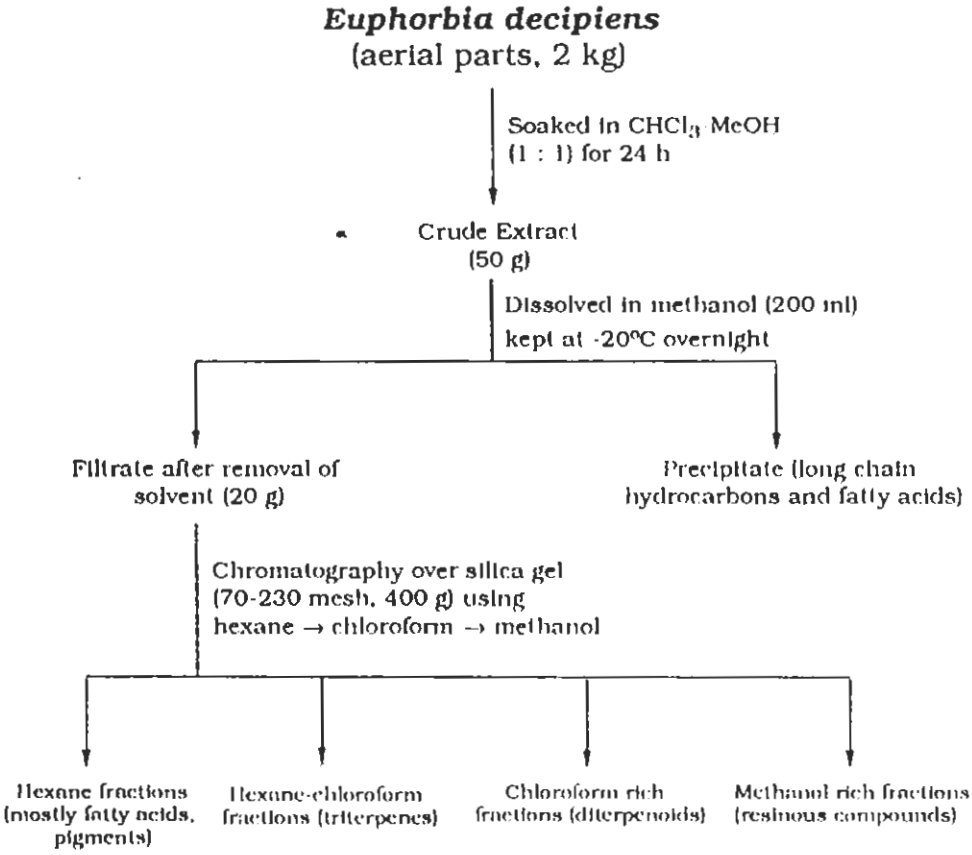
3.2.2 Extraction and Isolation

Air dried whole plant material (2 kg) was powdered and soaked in MeOH:$CHCl_3$ (1:1) at room temperature for 24 h. The extract was concentrated under reduced pressure, dissolved in a small amount of methanol and, after keeping in a freezer overnight, was filtered to remove long chain hydrocarbons (136, 137). The extract, after concentration (20 g), was chromatographed over a silica gel column (400 g, 70-230 mesh, Merck) using hexane (2 x 250 ml) with 25% gradient of chloroform upto 100% and then followed by 2% increasing of polarity upto 10% methanol in chloroform, and then upto 100% methanol with a 10% gradient in polarity. The initial fractions (upto 6) which were prominent in hexane, contained mostly of triterpenes, and the chloroform rich fractions contained mostly diterpenes. The diterpene

esters were isolated from the later fractions using repeated column chromatography (flash silica gel, 230-400 mesh) and preparative TLC (silica gel 60 GF_{254}) using hexane-ethyl acetate and chloroform-acetone alternat ely.

Decipinone and decipidone are the two major diterpenoids in *E. decipiens* which were separated from a white solid material, precipitated by using methanol from the chloroform-rich fractions (fractions 7-12). This mixture consisted of four compounds which was separated by column chromatography (flash silica gel, 230-400 mesh) using hexane-ethyl acetate (75:25) into two components, one visible under UV light on TLC (decipinone and isodecipinone) and other invisible (decipidone and isodecipidone). Each was separated into single spots by TLC (silica gel F_{254}) using chloroform-acetone (98:2).

The polar parts of the fractions, 7-12 were combined and loaded on a flash silica gel column (230-400 mesh) and eluted with hexane-chloroform (1:1) by increasing the polarity from 50% upto pure chloroform then to 10% acetone in chloroform with 1% gradient of polarity. The similar fractions were combined and subjected to repeated flash chromatography over silica gel using hexane-ethyl acetate for the purification of decipinone A, decipinol esters A and B, and karajinone A and B. The less polar fractions (6 and 7), obtained by hexane-chloroform (between 30-60%), consisted mostly of triterpenoids which were purified by repeated column chromatography and preparative TLC.



Scheme 3.1: Fractionations scheme of the chloroform-methanol extract of *Euphorbia decipiens*

3.2.3 Purification and Physical Constants of decipinone (79)

Decipinone (25 mg) was purified by preparative TLC (silica gel F_{254}) using chloroform-acetone (98 : 2) R_f 0.70, Yield (0.0013%), m.p. 246-249°C, $[\alpha]_D^{27}$=-21.3° ($CHCl_3$, c=0.19).

3.2.3.1 Crystallization of decipinone for X-ray Crystallography Analysis

The colourless plate-like crystals of **79** were grown from methanol at room temperature (low rate evaporation of the solvent). The crystals were specified and subjected for X-ray analysis.

3.2.3.2 X-Ray Structure Analysis of decipinone (79)

3.2.3.2.1 Crystal Data:

$C_{35}H_{42}O_{12}$, F.W.=654.69, orthorhombic, space group $P2_12_12$ (#18), lattice parameters: a=15.574(3), b=19.620(4), c=11.314(2) Å, V=3457.1(11) Å^3; D_{calc}=1.258 g cm^{-3}, Z=4, λ (Cu Kα)=1.5418 Å, μ=0.789 mm^{-1}, F(000)=1392, T=295(1) K.

3.2.3.2.2 Data Collection

Colourless plate-like crystals of **79** having approximate dimensions of 0.60 x 0.50 x 0.04 mm, were grown from methanol and mounted on a glass fiber. All measurements were made on an Enraf Nonius CAD-4 diffractometer with Cu-Kα radiation. Cell constants and an

orientation matrix for data collection, obtained from a least-squares refinement using the setting angles of 25 carefully centered reflections in the range $20.0 < \theta < 30.0^{o}$, corresponded to a primitive orthorhombic cell. Based on the systematic absences of : h00, h=2n+1, the space group was uniquely determined to be: $P2_12_12$ (#18). The data were collected at a temperature of 295 (1) K using the ω-2θ scan technique to a maximum 2θ value of 60.0^{o} with indices: h=0 to 17, k=-21 to 22 and l=0 to 12; Friedel pairs were not merged.

3.2.3.2.3 Data Reduction

Data reduction of the 5619 reflections which were collected, 5134 were unique (Rint=0.047). The intensities of three representative reflections were measured after every 200 reflections. Over the course of data collection, the standards did not show any sign of decay. The linear absorption coefficient, μ, for Cu-Kα radiation is 0.79 mm^{-1} which is sufficiently small, therefore, absorption correction was deemed unnecessary. The data were corrected for Lorentz and polarization effects.

3.2.3.2.4 Structure Solution and Refinement

The structure was solved by direct methods, (138) and expanded using Fourier techniques (139). The non-hydrogen atoms except phenyl C-atoms were refined anisotropically. The phenyl ring was disordered and its atoms were refined as constrained hexagons over two sites with partial occupancies and isotropic temperature factors. Hydrogen atoms were included at geometrically idealized positions with C-H and O-H

0.95 A° and were not refined. The final cycle of full-matrix least-squares refinement using F^2 was based on 4257 observed reflections ($I>2.0\,\delta(I)$) and 379 variable parameters and converged (largest parameter shift was 0.001 times its esd) with unweighted and weighted agreement factors R=0.076 (R=0.096 for all data) and wR=0.202, respectively, and goodness of fit, S=1.119. The weighting scheme was based on counting statistics. The maximum and minimum peaks in the final difference Fourier map corresponded to 0.30 and -0.34 $e^- Å^{-3}$, respectively. Neutral atom scattering factors were taken from Cromer and Waber (140). Anomalous dispersion effects were included in Fcalc (141), the values of $\Delta f'$ and $\Delta f''$ were those of Creagh and McAuley (142). The values for the mass attenuation coefficients are those of Creagh and Hubbell (143). All calculations for data reduction were performed using the TEXSAN (144), crystallogrphic software package of Molecular Structure Corporation and the refinement was carried out with the aid of SHELX93 (145). The absolute configuration could not be established by Flack (146) method; the Flack parameter was 0.3 (4). A full details on the XRD analysis of **79** have been submitted as supplementary material (see Appendix A).

3.2.3.3. Spectral Data of decipinone (79)

IR υ_{max}(KBr) cm^{-1}: 3500, 2950, 2910, 1740, 1720, 1630, 1600, 1580, 1450, 1230, 1020, 710, 600.

UV λ_{max} (MeOH) nm: 198.4, 227.2, 270.8.

EIMS *m/z* (rel. int.): 654 $[M]^+$ (1), 594 (1), 534 (1), 536 (1), 474 (1), 414 (1), 384 (12), 324 (12), 282 (26), 264 (57), 251 (24), 239 (27), 237 (27),

207 (38), 184 (10), 152 (13), 158 (71), 175 (48), 156 (56), 131 (52), 125 (47), 121 (12), 105 (100), 83 (73), 85 (65), 77 (23).

CIMS *m/z* (rel.Int.): 655 [M+1]$^+$ (6), 595 (25), 535 (50), 493 (8), 475 (12), 413 (14), 355 (12), 123 (58), 105 (16), 61 (100), 41 (34).

HREIMS *m/z*: 654.2698 (calcd 654.2676 for $C_{35}H_{42}O_{12}$).

^{1}H- and ^{13}C-NMR (in $CDCl_3$, 400 MHz and 75.4 MHz): See Table-2.1.

3.2.4 Purification of isodecipinone (84)

Isodecipinone (2 mg) was purified by preparative TLC (silica gel, F_{254}) from decipinone, using chloroform-acetone (98 : 2), R_f 0.65. Due to interchanging of this compound to decipinone (**79**) during the purification process, the physical constants could not be determined.

Yield 0.0001%.

3.2.4.1 Spectral Data of isodecipinone (84)

IR υ_{max} (KBr) cm^{-1}: 3480, 2920, 1740, 1720, 1630, 1600, 1450, 1370, 1270, 1230, 1020, 710, 600.

EIMS *m/z* (rel. int.): 654 $[M]^+$ ($C_{35}H_{42}O_{12}$) (1), 434 (1), 433 (5), 269 (6), 237 (12), 173 (9), 157 (7), 131 (12), 105 (100), 76 (13).

CIMS *m/z* (rel.int.): 655 $[M+1]^+$ (2), 595 (15), 535 (100), 423 (7), 475 (12),413 (16), 353 (11), 123 (30), 61 (95), 41 (58).

^{1}H-NMR (in $CDCl_3$, 400 MHz): See Table-2.2.

^{13}C-NMR (in $CDCl_3$, 125 MHz): δ 200.2, 170.3, 170.0, 169.5, 169.0, 165.0, 133.9, 129.6, 129.4, 129.3, 128.4, 126.6, 112.5, 88.6, 85.6, 80.6, 68.6, 67.8, 61.0, 51.5, 50.7, 44.1, 43.5, 42.0, 35.6, 25.9, 21.5, 21.3, 21.1, 20.8, 20.6, 14.5.

3.2.5 Purification and Physical Constants of decipidone (85)

Decipidone (24 mg) which was separated by column chromatography (silica gel, 230-400 mesh), using hexane-ethyl acetate (70:30), from decipinone, was resolved from its isomer, isodecipidone by repeated column chromatography over silica gel (230-400 mesh), using hexane-chloroform (4:6) upto 100% chloroform.

Yield 0.0012%, m.p. 186-188°C. $[\alpha]_D^{24}$= -23.8° ($CHCl_3$, c=0.48).

3.2.5.1 Spectral Data of decipidone (85)

IR υ_{max} (KBr) cm^{-1}: 3500, 2980, 2950, 1730, 1450, 1370, 1250, 1170, 1140, 1020, 750, 620.

UV λ_{max} (MeOH) nm: 198.5.

EIMS *m/z* (rel. int.): 620 $[M]^+$ (1), 560 (1), 500 (1), 384 (3), 282 (13), 264 (27), 239 (11), 228 (8), 185 (18), 175 (22), 158 (40), 156 (37), 132 (20), 131 (40), 125 (46), 71 (100), 69 (17), 60 (23).

CIMS *m/z* (rel. int.): 620 (100), 561 (100), 501 (100), 473 (40), 441 (60), 413 (50), 371 (33), 353 (75), 293 (50), 265 (15), 89 (20), 61 (47).

HREIMS *m/z* 620. 2789 (calcd. 620.2833 for $C_{32}H_{44}O_{12}$).

^{1}H- and ^{13}C-NMR (in $CDCl_3$, 400 MHz and 75.4 MHz): See Table-2.3.

3.2.6 Purification of isodecipidone (86)

Isodecipidone was obtained as a minor compound (2 mg) and purified from decipidone (**85**) by preparative TLC, using chloroform-acetone (98 : 2) R_f 0.68.

Yield 0.0001%.

3.2.6.1 Spectral Data of Isodecipidone (86)

CIMS *m/z* (rel.int.): 621 $[M+1]^+$ (81), 561 (100), 533 (12), 519 (35), 501 (100), 473 (35), 459 (29), 441 (41), 413 (50), 371 (26), 353 (52), 293 (41), 311 (19), 89 (14), 61 (24).

^{1}H-NMR (in $CDCl_3$,400 MHz): See Table-2.4.

3.2.7 Purification and Physical Constants of decipinone A (87)

Decipinone A (11 mg) was purified on preparative TLC (silica gel, F_{254}) using chloroform-acetone (92 : 8), R_f 0.40.

Yield 0.0006%, $[\alpha]_D^{27}$ = -9.1° ($CHCl_3$, c=0.22)

3.2.7.1 Spectral Data of decipinone A (87)

IR υ_{max} (KBr) cm^{-1}: 3500, 2980, 1730, 1630, 1580, 1370, 1240, 1110, 1020, 960, 730, 600.

UV λ_{max} (MeOH) nm: 199.8, 260.4.

EIMS *m/z* (rel.int.): 683 $[M]^+$ (2), 623 (3), 563 (1),493 (28), 392 (11), 282 (20), 281 (20), 264 (35), 255 (18), 254 (49), 251 (25), 239 (21), 158 (19), 131 (37), 125 (35), 124 (100), 106 (93), 85 (48), 83 (72),71 (76), 60 (10), 55 (15).

CIMS (CH_4) *m/z* (rel.int.) 683 (100), 623 (65), 563 (50), 493 (25), 124 (35), 89 (15), 61 (15).

HREIM *m/z* 683.2919 (calcd. 683.2942 for $C_{36}H_{45}O_{12}N$).

^{1}H- and ^{13}C-NMR (in $CDCl_3$, 500 MHz and 125 MHz): See Table-2.5.

3.2.8 Purification and Physical Constants of decipinol ester A (88)

Decipinol ester A (10 mg) was purified by preparative TLC (silica gel F_{254}) using chloroform-acetone (92:8) R_f 0.50.

Yield 0.0006%, m.p. 190-192°C, $[\alpha]_D^{27}$= -10° (in $CHCl_3$, c=0.08).

3.2.8.1 X-Ray Structure Analysis of decipinol ester (88)

3.2.8.1.1 Crystal Data

The colourless plate-like crystals were grown from methanol at room temperature. $C_{33}H_{40}O_{11}$, F.W.=612.67, Crystal dimensions: 0.50 X 0.48 X 0.22 mm, Orthorhombic, space group $P2_12_12_1$ (#19), Cell determination (2θ range): 25 (40.0-50.0°), Lattice parameters: a=8.893 (4) Å, b=17.509 (2) Å, c=20.276 (4) Å, V=3157 (1) Å; D_{calc}=1.29 g/cm^3, Z=4, μ (CuKα)=8.05 cm^{-1}.

3.2.8.1.2 Data Collection

A colourless plate crystal of **88** having approximate dimensions of 0.50 X 0.48 X 0.22 mm was mounted on a glass fiber. All measurements were made on an Enraf-Nonius CAD-4 diffractometer with graphite monochromated Cu-Kα (λ=1.54178 Å) radiation. Cell constants and an orientation matrix for data collection, obtained from a least-squares refinement, using the setting angles of 25 carefully centered reflections in the range of 40.00 <2θ <50.00° corresponded to a primitive orthorhombic cell with dimensions: a=8.893 (4)Å, b=17.509(2)Å,

c=20.276(4)Å, V=3157(1)Å. For Z=4 and F.W. =612.67, the calculated density is 1.29 g/cm^3. The systematic absences of h00: h≠2n, 0k0: k≠2n, 001: l≠2n, uniquely determine the space group to be $P2_12_12_1$ (≠19). The data were collected at a temperature of 22±1°C, using the ω scan technique to a maximum 2θ value of 135.8°.

3.2.8.1.3 Data Reduction

A total of 3246 unique reflections were collected. Over the course of data collection, the standards decreased by 2.0%. A linear correction factor was applied to the data to account for this phenomenon. The linear absorption coefficient, μ, for Cu-Kα radiation is 8.05 cm^{-1}. An absorption correction was deemed unnecessary. The data were corrected for Lorentz and polarization effects.

3.2.8.1.4 Structure Solution and Refinement

The structure was solved by direct methods (147) and expanded using Fourier techniques (139). The non-hydrogen atoms were refined anisotropically. The phenyl ring C-atoms were constrained as regular hexagon. Hydrogen atoms were included at geometrically idealized positions. The final cycle of full-matrix least-squares refinement using F^2 coefficients with the aid of SHELXL97 (148) was based on 1266 observed reflections ($I > 2.00\sigma(I)$) and 395 variable parameters and converged (largest parameter shift was 0.023 times its esd) with unweighted and weighted agreement factors: R=0.0671 and wR =0.1386. The standard deviation of an observation of unit weight was 1.021. The weighting scheme was based on counting statistics. The maximum and

minimum peaks on the final difference Fourier map corresponded to 0.163 and -0.152 $e^-/Å^3$, respectively. An absolute configuration could not be established. However, the Flack parameter (146) was 0.1 (7) for the structure reported here; its value being 0.5 (8) for the inverted structure.

Neutral atom scattering factors were taken from Cromer and Waber (140). Anomalous dispersion effects were included in Fcals (141). The values for $\Delta f'$ and $\Delta f''$ were those of Creagh and McAuley (142). The values for the mass attenuation coefficients are those of Creagh and Hubbell (143). All calculations for data reduction were performed using the teXsan (149) crystallographic software package of Molecular Structure Corporation.

3.2.8.2 Spectral Data of decipinol ester A (88)

IR υ_{max} (KBr) cm^{-1}: 3450, 2900-3000, 1720, 1730, 1640, 1450, 1370, 1270, 1230, 1100,1020, 870, 710.

UV λ_{max} (MeOH) nm: 199.0, 227.6, 270.0.

EIMS *m/z* (rel.int.): 594 $[M-H_2O]^+$ (1), 552 $[M-HOAc]^+$ (1), 492 (2), 432 (4), 430 (14), 387 (8), 370 (22), 328 (15), 310 (20), 299 (9), 282 (13), 267 (13), 253 (10), 239 (25), 218 (13), 197 (10), 175 (25), 173 (22), 145 (10), 131 (15), 105 (100), 60 (3), 55 (7).

CIMS (CH_4) (rel.int.): 612 $[M]^+$ (13), 552 (100), 492 (40), 431 (45), 371 (43), 311 (35),123 (23), 105 (15), 61 (45).

^{1}H- and ^{13}C-NMR (in $CDCl_3$, 500 MHz and 75.4 MHz): See Table-2.6.

^{1}H- and ^{13}C-NMR (in CD_3OD, 500 MHz and 125 MHz): See Table-2.7.

3.2.9 Purification and Physical Constants of decipinol ester B (90)

Decipinol ester B was obtained as minor compound (6 mg) and purified by repeated column chromatography (silica gel 230-400 mesh) using hexane-chloroform with gradient (5%) of polarity to chloroform then chloroform-acetone (90-10). Purity was checked on TLC using chloroform-acetone (95:5) R_f 0.49.

Yield 0.0003%, m.p. 232-234°C, $[\alpha]_D^{24}$= + 12.4° (in $CHCl_3$, c=0.1)

3.2.9.1 Spectral Data of decipinol ester B (90)

IR υ_{max}($CHCl_3$) cm^{-1}: 3450, 2900, 2840, 1736, 1728, 1712, 1640, 1450, 1370, 1280, 1150, 1100, 1020, 990, 970, 900, 600, 520.

UV λ_{max} (MeOH) nm: 203.

EIMS m/z (rel.int.): 578 (0.4), 560 (1), 518 (1), 490 (1), 458 (2), 430 (9), 413 (2), 399 (9), 370 (21), 352 (2), 328 (14), 310 (28), 299 (10), 282 (12), 267 (16), 251 (17), 239 (29), 225 (11), 218 (21), 207 (15), 191 (19), 175 (46), 173 (53), 159 (19), 149 (26), 131 (43), 125 (39), 95 (29), 81 (30), 71 (100), 57 (35), 55 (30).

^{1}H- and ^{13}C-NMR (in $CDCl_3$, 500 MHz, 75.4 MHz): See Table-2.8.

3.2.10 Purification and Physical Constants of karajinone A (91)

Karajinone A (10 mg) was purified by preparative TLC using chloroform-acetone (92:8) R_f 0.62.

Yield 0.0005%, $[\alpha]_D^{27}$= -7° (in $CHCl_3$, c=0.2)

3.2.10.1 Spectral Data of karajinone A (91)

IR υ_{max}($CHCl_3$) cm^{-1}: 3000, 1740, 1730, 1605, 1460, 1380, 1320, 1280, 1120, 1080, 1030, 980, 610.

UV λ_{max} (MeOH) nm: 198.2, 227.4, 270.8.

EIMS *m/z* (rel.int.): 626 (3), 594 (1), 566 (9), 233 (37), 173 (100), 145 (12), 133 (10), 131 (8), 121 (2), 125 (9), 105 (94), 77 (11).

CIMS *m/z*: 654 ($C_{35}H_{42}O_{12}$) $[M]^+$, 594 (100), 534, 474, 431, 371, 311, 173, 123, 105.

HREIMS m/z: 626.2706 ($C_{34}H_{42}O_{11}$) $[M-CO]^+$, 594.2441 ($C_{33}H_{38}O_{10}$) $[M-HOAc]^+$.

^{1}H- and ^{13}C-NMR (in $CDCl_3$, 500 MHz and 75.4 MHz): See Table-2.9.

3.2.11 Purification and Physical Constants of karajinone B (94)

Karajinone B (11 mg) was purified by preparative TLC (silica gel F_{254}) using chloroform-acetone (92:8) R_f 0.67.

Yield 0.0006%, $[\alpha]_D^{27}$= -1.8° (in $CHCl_3$, c=0.22)

3.2.11.1 Spectral Data of karajinone B (94)

IR υ_{max} (KBr) cm^{-1}: 3450, 2980, 1740, 1720, 1650, 1620, 1600, 1570, 1540, 1520, 1470, 1370, 1270, 1245, 1110, 1100, 1070, 1020, 710, 600.

UV λ_{max} (MeOH) nm: 200.0, 227.6, 270.8.

EIMS *m/z* (rel.int.): 584 (4), 524 (21), 342 (7), 282 (7), 239 (6), 233 (34), 175 (21), 173 (100), 145 (21), 133 (11), 105 (21), 60 (10).

CIMS *m/z*: 612 ($C_{33}H_{40}O_{11}$) $[M]^+$, 583, 552 (100), 534, 492, 431, 371, 123, 105, 61, 41.

HREIMS *m/z*: 584.2559 ($C_{32}H_{40}O_{10}$) $[M-CO]^+$, 552.2364 ($C_{31}H_{36}O_9$) $[M-HOAc]^+$

^{1}H- and ^{13}C-NMR (in $CDCl_3$, 500 MHz and 75.4 MHz):See Table-2.10.

3.2.12 Purification of β-sitosterol (95)

From the 6th fraction of the first column chromatographic step (50% hexane-chloroform) **95** was purified by crystallization from methanol.

3.2.12.1 Spectral Data of β-sitosterol (95)

^{1}H-NMR (in $CDCl_3$, 400 MHz): δ 0.67 (s, 3H-18), 1.00 (s, 3H-19), 0.91 (d, *J*=6.6 Hz, 3H-21), 0.84 (d, J=7.3 Hz, 3H-26, 3H-27), 0.81 (t, *J*=6.5 Hz, 3H-29), 3.51 (m , H-3), 5.34 (m, H-6).

EIMS *m/z* (rel.int.): 414 $[M]^+$ (100%), 399 $[M\text{-}CH_3]^+$ (16), 396 $[M\text{-}H_2O]^+$ (19), 329 (14), 303 (21), 273 (10), 213 (10), 161 (10), 145 (12), 135 (9), 119 (10), 107 (18), 95 (17), 81 (17), 55 (21).

3.2.13 Purification of cycloeucalenol (96)

Cycloeucalenol was obtained from the 7th fraction of the first chromatographic step with polarity of 60% hexane in chloroform and was purified by preparative TLC using chloroform-methanol (60 ml : 6 drop). Cycloeucalenol (**96**) and obtusifoliol (**97**) were obtained as a mixture and identified by spectral data.

3.2.13.1 Spectral Data of cycloeucalenol (96)

^{1}H-NMR (in $CDCl_3$, 400 MHz): δ 0.14 (d, *J*=4.2 Hz, H-19), 0.38 (br d, *J*=2.9 Hz, H-19'), 0.89 (s, 3H-32), 0.92 (d, J=6.5 Hz, 3H-21), 0.97 (s, 3H-18), 0.99 (d, J=6.2 Hz, 3H-30), 1.02 (d, J=6.8 Hz, 3H-26), 1.03 (d, J=6.8 Hz, 3H-27), 2.23 (sep, J=6.9 Hz, H-25), 3.20 (ddd, J=5, 9.8, 10 Hz, Hα-3), 4.65 (br s, H-28), 4.71 (br s, H-28').

^{13}C-NMR (in $CDCl_3$, 75.4 MHz): δ 30.8 (C-1), 35.1 (C-2), 76.1 (C-3), 44.6 (C-4), 43.4 (C-5), 24.7 (C-6), 25.7 (C-7), 47.1 (C-8), 23.6 (C-9), 29.6 (C-10), 27.1 (C-11), 32.9 (C-12), 45.4 (C-13), 49.9 (C-14), 35.4 (C-15), 28.2 (C-16), 52.3 (C-17), 17.8 (C-18), 27.2 (C-19), 36.2 (C-20), 18.4 (C-21), 35.1 (C-22), 31.3 (C-23), 156.8 (C-24), 33.9 (C-25), 22.0 (C-26), 21.9 (C-27), 106.0 (C-28), 14.4 (C-30), 19.2 (C-32).

EIMS *m/z* (rel.int.): 426 $[M]^+$ (56), 411 $[M-Me]^+$ (70), 408 $[M-H_2O]^+$ (24), 393 (16), 300 (11), 283 (4), 175 (20), 55 (85), 69 (100).

3.2.14 Purification of obtusifoliol (97)

Obtusifoliol was obtained together with (96) and identified by comparison of the spectral data with standard compound (99-101).

3.2.14.1 Spectral Data of obtusifoliol (97)

^{1}H-NMR (in $CDCl_3$, 400 MHz): δ 0.71 (s, 3H-18), 0.89 (s, 3H-32), 0.93 (d, *J*=6.5 Hz, 3H-30), 0.97 (s, H-19), 1.00 (d, *J*=6.2 Hz, 3H-21), 1.01 (d, J=6.8 Hz, 3H-26), 1.03 (d, *J*=6.8 Hz, 3H-27), 3.10 (ddd, *J*= 5.0, 9.8, 10.0 Hz, H-3), 4.66 (br s, H-28), 4.72 (br s, H-28').

^{13}C-NMR (in $CDCl_3$, 75.4 MHz): δ 35.1 (C-1), 31.3 (C-2), 76.6 (C-3), 39.3 (C-4), 47.2 (C-5), 20.8 (C-6), 28.2 (C-7), 133.7 (C-8), 134.7 (C-9), 36.4 (C-10), 21.9 (C-11), 25.6 (C-12), 44.6 (C-13), 49.9 (C-14), 31.4 (C-15), 31.2 (C-16), 50.5 (C-17), 15.8 (C-18), 18.8 (C-19), 36.5 (C-20), 18.4 (C-21), 35.1 (C-22), 30.8 (C-23), 156.9 (C-24), 33.9 (C-25), 21.9 (C-26), 22.01 (C-27), 105.9 (C-28),15.1 (C-30), 24.5 (C-32).

EIMS *m/z* (rel.int.): 426 $[M]^+$ (20), 393 (16), 327 (9), 259 (8), 245 (20), 233 (10), 173 (19), 159 (21), 69 (100).

3.2.15 Purification of cycloart-23-ene-3,25-diol (98)

Cycloart-23-ene-3,25-diol was separated from the non polar fraction eluted with 60% hexane in chloroform from the initial column chromatography and purified by preparative TLC (silica gel F_{254}) using chloroform-methanol (60 ml : 6 drop).

3.2.15.1 Spectral Data of cycloart-23-ene-3,25-diol (98)

^{1}H-NMR (in $CDCl_3$, 500 MHz): δ 0.31 (d, *J*=4.1 Hz, H-19), 0.53 (d, *J*=4.0 Hz, H-19'), 0.79 (s, 3H-18), 0.84 (d, *J*=6.5 Hz, 3H-21), 0.86 (s, 3H-30), 0.95 (br s, 3H-28, 3H-29), 1.29 (br s, 3H-26, 3H-27), 3.26 (dd, *J*=4.3, 7.0 Hz, H-3), 5.58 (br s, H-23, H-24).

EIMS *m/z* (rel. int.): 442 $[M]^+$ ($C_{30}H_{50}O_2$) (4), 424 $[M-H_2O]^+$ (20), 409 (20), 302 (33), 255 (22), 203 (45), 189 (14), 176 (60), 173 (100), 161 (40), 135 (57), 119 (56), 109 (98), 107 (90), 95 (97), 81 (97), 69 (98), 55 (99).

3.2.16 Purification of 24-methylenecycloartan-3β-ol (99)

The purification procedure is the same as described for cycloart-23-ene-3,25-diol.

3.2.16.1 Spectral Data of 24-methylenecycloartan-3β-ol (99)

^{1}H-NMR (in $CDCl_3$, 400 MHz): δ 0.32 (d, *J*=4.1 Hz, H-19), 0.54 (d, *J*=4.0 Hz, H-19'), 0.80 (s, 3H-30), 0.87 (d, *J*=6.4 Hz, 3H-21), 0.88 (s, 3H-28), 0.96 (s, 3H-18, 3H-29), 1.01 (d, *J*=6.8 Hz, 3H-26), 1.02 (d, *J*=6.9, 3H-27), 3.27 (dd, *J*=4.5, 11.3 Hz, H-3), 4.66 (br s, H-31), 4.70 (br s, H-31').

EIMS *m/z* (rel. int.): 440 $[M]^+$ ($C_{31}H_{52}O$) (72), 422 $[M-H_2O]^+$ (93), 407 (61), 379 (25), 300 (83), 219 (12), 203 (35), 175 (50), 161 (31), 147 (38), 135 (53), 121 (52), 109 (63), 107 (58), 95 (87), 83 (70), 69 (100), 55 (56).

3.3 Part B: Phytochemical Investigation of *Euphorbia teheranica*

3.3.1 Plant material

The aerial parts of the plant were collected from Tehran in July 1996 and identified by Prof. Dr. M. Sanei Chariat Pannahi, Karaj Agriculture College, University of Tehran, Iran. A voucher specimen (No. 2256) is deposited in the herbarium of the mentioned college. The plant material was dried under shade and powdered to yield 1.2 kg.

3.3.2 Extraction and Isolation

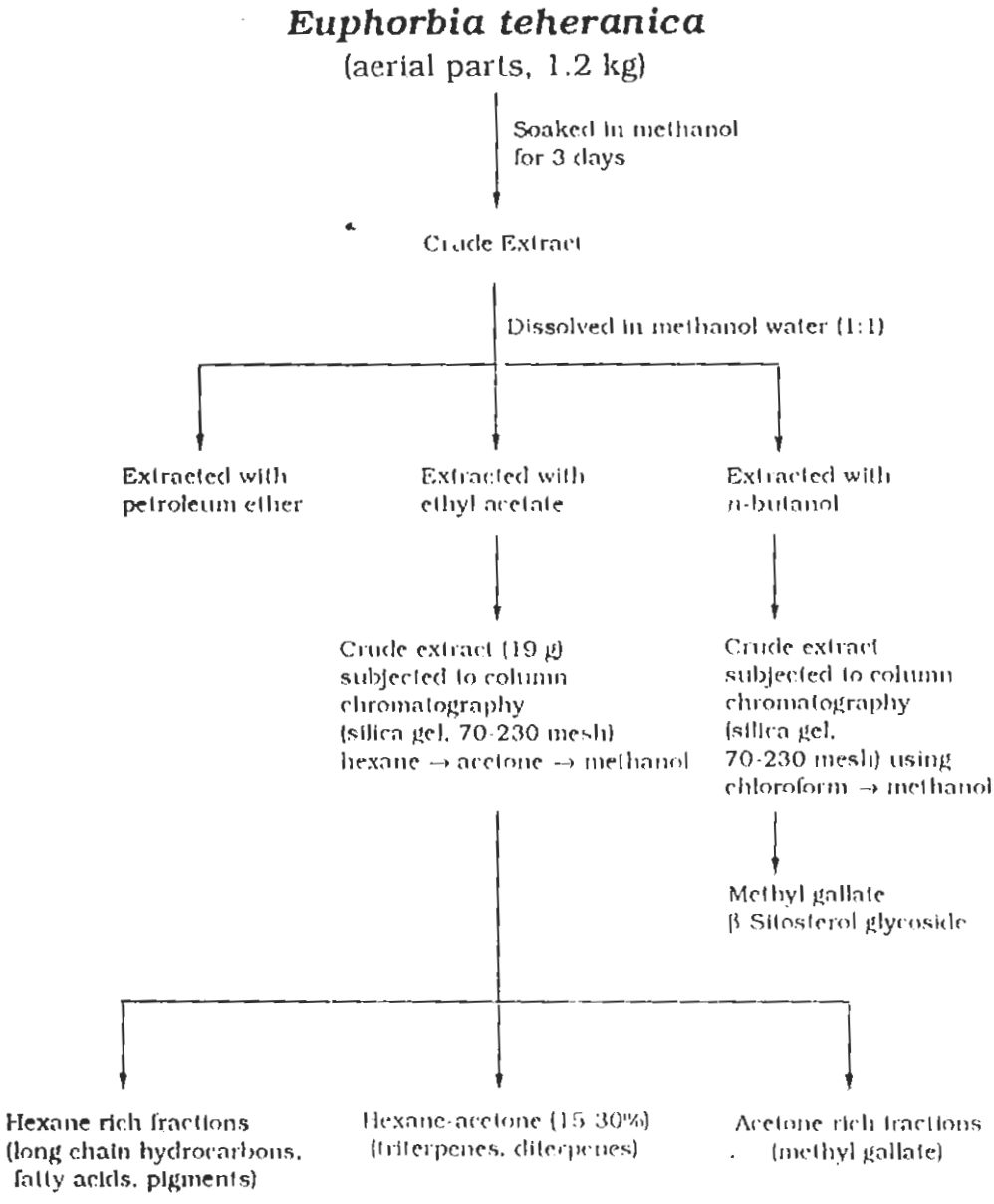
The powder of the plant (1.2 kg) was extracted with methanol, at room temperature for 3 days. Removal of the solvent under reduced pressure gave a syrup which was dissolved in a mixture of methanol:water (1:1) and subsequently extracted with hexane, ethyl acetate and finally butanol. The ethyl acetate fraction after concentration (19 g), was subjected to column chromatography over silica gel (400 g, 70 230 mesh), using hexane (1 L) with a 2.5% increasing of polarity upto 100% acetone (250 ml each fraction), and then pure methanol.

The similar fractions obtained by elution between 20 to 30% acetone in hexane after monitoring with TLC were combined and subjected to repeated flash chromatography (silica gel 230-400 mesh), using 50% hexane in chloroform with increasing polarity upto 100% chloroform and then to acetone. Repeated preparative TLC (silica gel F_{254}) using

1% methanol or 10% acetone in chloroform led to purify the diterpene esters, tehranone A (**100**) and B (**101**) and tehranol ester (**102**).

Methyl gallate (**105**) was seperated from a 50% hexane - acetone fraction obtained from initial column chromatographic step and purified by flash chromatography using chloroform:acetone (4:1).

Two triterpenes betulin (**106**) and erythrodiol (**107**) were obtained as a mixture and purified by impregnated column chromatography (10% $AgNO_3$ on flash silica gel) using chloroform. β-Sitosterol glycoside (**109**) was precipitated from methanol rich fractions as a pure compound and oleanolic acid (**108**) was obtained from the fractions prominent in chloroform by repeated column chromatography.



Scheme-3.2: Fractionation scheme of the methanol extract of *Euphorbia teheranica*.

3.3.3 Purification and Physical Constants of tehranone A (100)

Tehranone A (18 mg) was purified by repeated preparative TLC (silica gel F_{254}) using chloroform-acetone (9:1) R_f 0.73.

Yield 0.0015%, m.p. 233-234°C. $[\alpha]_D^{27}$= +49.3° (in $CHCl_3$, c=0.36).

3.3.3.1 Spectral Data of tehranone A (100)

IR ($CHCl_3$) υ_{max}: 2980, 1740, 1370, 1260, 1170, 1140, 1025 cm^{-1}.

UV (MeOH) λ_{max} nm: 276, 230, 201.

EIMS *m/z* (rel.int.): 693 $[M+1]^+$, 633 (3), 573 (2), 513 (2), 488 (2), 471 (2), 343 (18), 250 (12), 249 (70), 247 (11), 235 (14), 207 (11), 205 (20), 191 (40), 181 (20), 148 (21), 105 (29), 85 (37), 71 (11), 57 (100).

CIMS *m/z* : 691 $[M-1]^+$, 633 $[M+1-HOAc]^+$, 573 $[M+1-2xHOAc]^+$, 531 $[M+1-HOAc-C_5H_{10}O_2]^+$.

HREIMS *m/z*: 633.2889 $[M+1-HOAc]^+$ (calcd. for $C_{33}H_{45}O_{12}$, 633.2911).

^{1}H- and ^{13}C-NMR (in $CDCl_3$, 500 and 75.4 MHz): See Table-2.11.

3.3.4 Purification and Physical Constants of tehranone B (101)

Tehranone B (4 mg) was purified by preparative TLC (silica gel F_{254}) using chloroform-acetone (9:1) R_f 0.38.

Yield 0.0003%, m.p. 202-203°C, $[\alpha]_D^{20}$= +25° (in $CHCl_3$, c=0.08).

3.3.4.1 Spectral Data of tehranone B (101)

IR (KBr) υ_{max}: 3450 (H_2O), 2980, 2950, 1740, 1580, 1460, 1420, 1370, 1280, 1220, 1160, 1130, 1110, 1080, 1020, 960, 920, 740, 700 cm^{-1}.

UV (MeOH) λ_{max} nm: 263, 219, 200.

EIMS *m/z* (rel.int.): 755 $[M]^+$, 696 (14), 654 (17), 610 (8), 552 (3), 510 (4), 406 (15), 364 (17), 249 (51), 191 (44), 149 (14), 124 (100), 106 (41), 85 (19), 57 (62), 42 (79).

HREIMS *m/z:* 755.3092 (calcd. for $C_{39}H_{49}O_{14}N$, 755.3153).

^{1}H- and ^{13}C-NMR (in $CDCl_3$, 500 and 75.4 MHz): See Table-2.12.

3.3.5 Purification and Physical Constants of tehranol ester (102)

Tehranol ester (14 mg) was purified by preparative TLC (silica gel F_{254}) using chloroform-acetone (9:1) R_f 0.34.

Yield 0.0012%, m.p. 183-184°C, $[\alpha]_D^{27}$= -48.8° (in $CHCl_3$, c=0.09).

3.3.5.1 Spectral Data of tehranol ester (102)

IR ($CHCl_3$) υ_{max}: 2980, 2400, 1730, 1590, 1360, 1270, 1110, 1010, 980, 600, 500 cm^{-1}.

UV (MeOH) λ_{max} nm: 264, 219, 201.

EIMS *m/z* (rel.int.): 697 $[M]^+$, 596 (2), 407 (8), 364 (13), 291 (10), 264 (7), 147 (24), 124 (68), 106 (100), 85 (2), 78 (22).

HREIMS *m/z:* 697.2758 (calcd. for $C_{36}H_{43}O_{13}N$, 697.2734).

^{1}H- and ^{13}C-NMR (in $CDCl_3$, 500 and 75.4 MHz): See Table-2.13.

^{1}H- and ^{13}C-NMR (in C_6D_6, 400 and 75.4 MHz): See Table-2.14.

3.3.6 Purification and Physical Constants of methyl gallate (105)

Methyl gallate (90 mg) was separated from a 50% hexane-acetone fraction, obtained from initial column chromatographic step. It was purified by column chromatography (silica gel, 230-400 mesh) using chloroform-acetone (4:1). **105** also was found as a major compound (910 mg) in the butanol fraction which was loaded on a column (silica gel 70-230 mesh) and eluted with chloroform → methanol.

Yield (0.1%), m.p. 196-198°C.

3.3.6.1 Spectral Data of methyl gallate (105)

IR (KBr) υ_{max}: 3500, 3300, 1690, 1610, 1530, 1460, 1435, 1310, 1250, 1190, 1040, 1000, 770, 740, 640, 550 cm^{-1}.

^{1}H-NMR (in CD_3COCD_3, 400 MHz): δ 8.27 (br s, 3H), 7.12 (s, 2H), 3.78 (s, 3H).

EIMS *m/z* (rel.int.): 184 $[M]^+$ (68), 153 $[M-OMe]^+$ (100), 125 $[M-CO_2Me]^+$ (15), 79 (8), 53 (2), 51 (5).

3.3.7 Purification of betulin (103)

Betulin (34 mg) was obtained from the fractions, eluted with hexane-acetone (90:10 to 80:20) in the initial column chromatographic step and purified by silver nitrate impregnated silica gel column chromatography (10% $AgNC_3$ on flash silica gel, 230-400 mesh) using chloroform as eluent.

Yield 0.017%.

3.3.7.1 Spectral Data of betulin (106)

^{1}H-NMR (in $CDCl_3$, 400 MHz): δ 0.75, 0.81, 0.96, 0.97, 1.01 (s, each 3H, Me-23, Me-24, Me-25, Me-26, Me-27), 1.67 (br d, J=0.6 Hz, Me-30), 3.17 (dd, *J*=5.1, 10.9 Hz, H-3), 3.31 (dd, *J*=0.3, 11.0 Hz, H-28), 3.78 (dd, *J*=1.9, 10.9 Hz, H-28'), 4.56 (m, H-29), 4.66 (br d, *J*=2.3 Hz, H-29').

^{13}C-NMR (in $CDCl_3$, 75.4 MHz): δ 38.8 (C-1), 27.5 (C-2), 79.0 (C-3), 38.9 (C-4), 55.4 (C-5), 18.4 (C-6), 34.3 (C-7), 40.1 (C-8), 50.5 (C-9), 37.4 (C-10), 20.9 (C-11), 25.3 (C-12), 37.2 (C-13), 42.8 (C-14), 27.1 (C-15), 29.2 (C-16), 47.8 (C-17), 47.8 (C-18), 48.8 (C-19), 150.4 (C-20), 29.8 (C-21), 34.0 (C-22), 28.0 (C-23), 15.4 (C-24), 16.1 (C-25), 16.0 (C-26), 14.8 (C-27), 60.6 (C-28), 109.8 (C-29), 19.1 (C-30).

EIMS *m/z* (rel. int.): 442 (55) $[M]^+$ ($C_{30}H_{50}O_2$), 427 (15) $[M-CH_3]^+$, 424 (52) $[M-H_2O]^+$, 411 (45) $[M-CH_2OH]^+$,409 (39), 381 (12), 315 (15), 302 (41), 373 (24), 234 (27), 203 (83), 189 (61), 175 (60), 161 (36), 121 (67), 95 (100), 81 (72), 55 (51).

3.3.8 Purification of erythrodiol (107)

Erythrodiol (28 mg) was separated along with **106** and purified by the help of silver nitrate impregnated column chromatography (see 3.3.7).

Yield 0.014%.

3.3.8.1 Spectral Data of erythrodiol (107)

^{1}H-NMR (in $CDCl_3$, 400 MHz): δ 0.77 (s, 3H-24), 0.86, 0.87 (s, 3H-29, 3H-30), 0.92, 0.93 (s, 3H-25, 3H-26), 0.98 (s, 3H-23), 1.15 (s, 3H-27), 3.20 (d, J=11 Hz, H-28), 3.53 (d, J=11 Hz, H-28'), 3.21 (dd, *J*=4.5, 11.2 Hz, H-3), 5.17 (t , *J*=3.6 Hz, H-12).

^{13}C-NMR (in $CDCl_3$, 75.4 MHz): δ 38.6 (C-1), 27.2 (C-2), 79.0 (C-3), 38.8 (C-4), 55.1 (C-5), 18.4 (C-6), 32.6 (C-7), 39.8 (C-8), 47.6 (C-9), 36.9 (C-10), 23.5 (C-11), 122.4 (C-12), 144.2 (C-13), 41.8 (C-14), 25.6 (C-15), 22.0 (C-16), 36.9 (C-17), 42.3 (C-18), 46.5 (C-19), 30.9 (C-20), 34.1 (C-21), 31.0 (C-22), 28.1 (C-23), 15.6 (C-24), 15.5 (C-25), 16.6 (C-26), 25.9 (C-27), 69.7 (C-28), 33.2 (C-29), 33.6 (C-30).

EIMS *m/z* (rel. int): 442 (8) $[M]^+$ ($C_{30}H_{50}O_2$), 409 (9), 234 (32), 216 (14), 207 (15), 204 (28), 203 (100), 189 (11), 119 (11), 107 (10), 95 (14), 81 (12), 69 (17).

3.3.9 Purification of oleanolic acid (108)

Oleanolic acid (2 mg) was separated from the fractions of the first chromatographic step which were eluted with 30-40% acetone in hexane. Purification was carried out using repeated column chromatography using chloroform with 1% gradient of acetone upto 20% acetone in chloroform.

3.3.9.1 Spectral Data of oleanolic acid (108)

^{1}H-NMR (in $CDCl_3$, 500 MHz): δ 0.74 (s, 3H), 0.76 (s, 3H), 0.89 (s, 3H), 0.90 (s, 3H), 0.91 (s, 3H), 0.96 (s, 3H), 1.12 (s, 3H), 2.80 (dd, J=3.8, 13.5 Hz, H-18), 3.20 (dd, J=4, 11 Hz, H-3), 5.26 (t, J=3.5 Hz, H-12).

EIMS *m/z* (rel.int.): 456 $[M]^+$ ($C_{30}H_{48}O_3$) (16), 411 $[M-CO_2H]^+$ (3), 248 (100), 207 (68), 204 (43), 203 (79), 189 (54).

3.3.10 Purification of β-sitosterol glycoside (109)

β-Sitosterol glycoside was precipitated from the acetone rich fractions of the first chromatographic step and further purified from hot methanol.

3.3.10.1 Spectral Data of β-sitosterol glycoside (109)

^{1}H-NMR (in DMSO-d_6, 400 MHz): δ 0.65 (s, 3H-18), 0.80 (t, J=6.5 Hz, 3H-29), 0.82 (d, J=7.3 Hz, 6H, Me-26, Me-27), 0.90 (d, J=6.2 Hz, 3H-21), 0.99 (s, 3H-19), 4.20 (d, J=7.8 Hz, H-1'), 5.32 (m, H-6).

^{13}C-NMR (in DMSO-d_6, 75.4 MHz): δ 36.8 (C-1), 29.3 (C-2), 77.0 (C-3), 38.3 (C-4), 140.5 (C-5), 121 (C-6), 31.4 (C-7), 31.5 (C-8), 49.7 (C-9), 36.2 (C-10), 20.6 (C-11), 39.4 (C-12), 41.8 (C-13), 56.2 (C-14), 23.8 (C-15), 27.7 (C-16), 55.4 (C-17), 11.7 (C-18), 19.1 (C-19), 35.5 (C-20), 18.6 (C-21), 33.4 (C-22), 25.6 (C-23), 45.2 (C-24), 28.8 (C-25), 18.9 (C-26), 18.9 (C-27), 22.6 (C-28), 11.7 (C-29), 100.8 (C-1'), 73.5 (C-2'), 76.7, 76.8 (C-3', C-5'), 70.2 (C-4'), 62.2 (C-6').

3.4 Part C: Chemical Investigation of the Essential Oil of *Zataria multiflora*

3.4.1 Plant Material

The aerial parts of the plant, *Zataria multiflora* were collected from Quetta in 1996 and identified by Dr. Rasool Bakhsh Tareen at the Department of Botany, University of Baluchistan, Quetta, Pakistan. A voucher specimen has been deposited in the herbarium of the Botany Department of Baluchistan University with a Herbarium No. 367.

3.4.2 Extraction of the Essential Oil

The dried plant material, mostly leaves and flowers after crushing (560 g), was subjected to a Clevenger-type hydrodistillation apparatus and distilled with water for 5h. The oil was separated as a light yellowish layer with a 2.2% w/w yield. The oil was dried over anhydrous sodium sulfate and stored in a freezer till the time of analysis.

3.4.3 Gas Chromatography

Gas chromatography using FID (flame ionization detector), was carried out on a Shimadzu gas chromatograph, GC-9A equipped with a C-R6A integrator, and an SE-30 column (30 m X 0.25 mm and 0.25 μm film thickness). After some experiments the optimum condition for analysis was obtained as : column temperature was, 10 min. at 60°C then raised to 220°C at 4°C/min. injector and detector temperature were set at 270°C, and the carrier gas was N_2 with a 1 ml/min. flow rate. 1 μ lit. of

a 10% v/v solution of the essential oil in ether with a split ratio of 1:30 was injected.

Figure 3.1 and 3.2 show the GC chromatograms of the oil. The concentration of the components were calculated by means of area normalization, which was obtained by the C-R6A integrator, without correction factor.

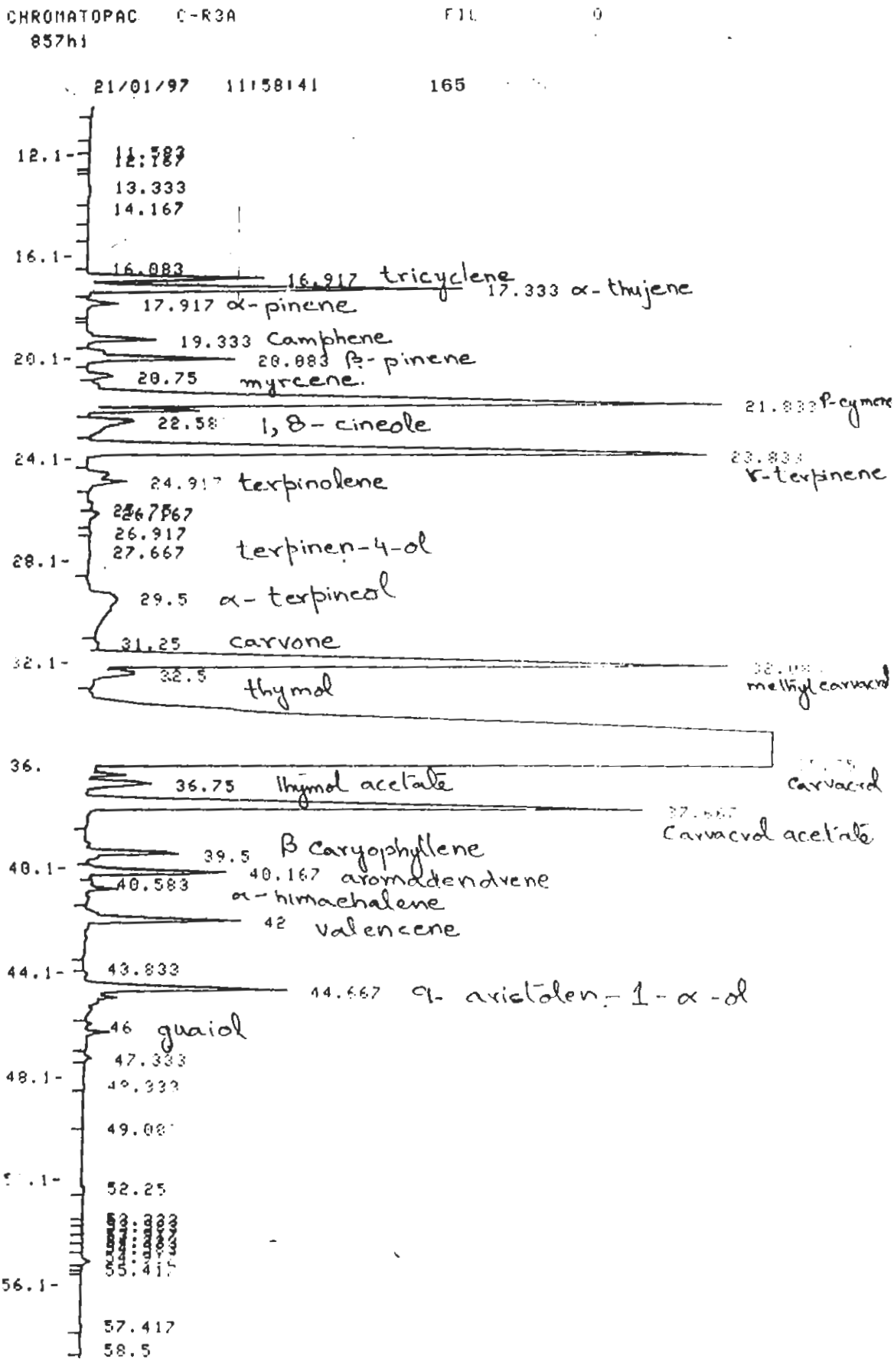


Figure 3. 1: The GC chromatogram of the essential oil of *Zataria multiflora* Boiss. on an SE 30 capillary column

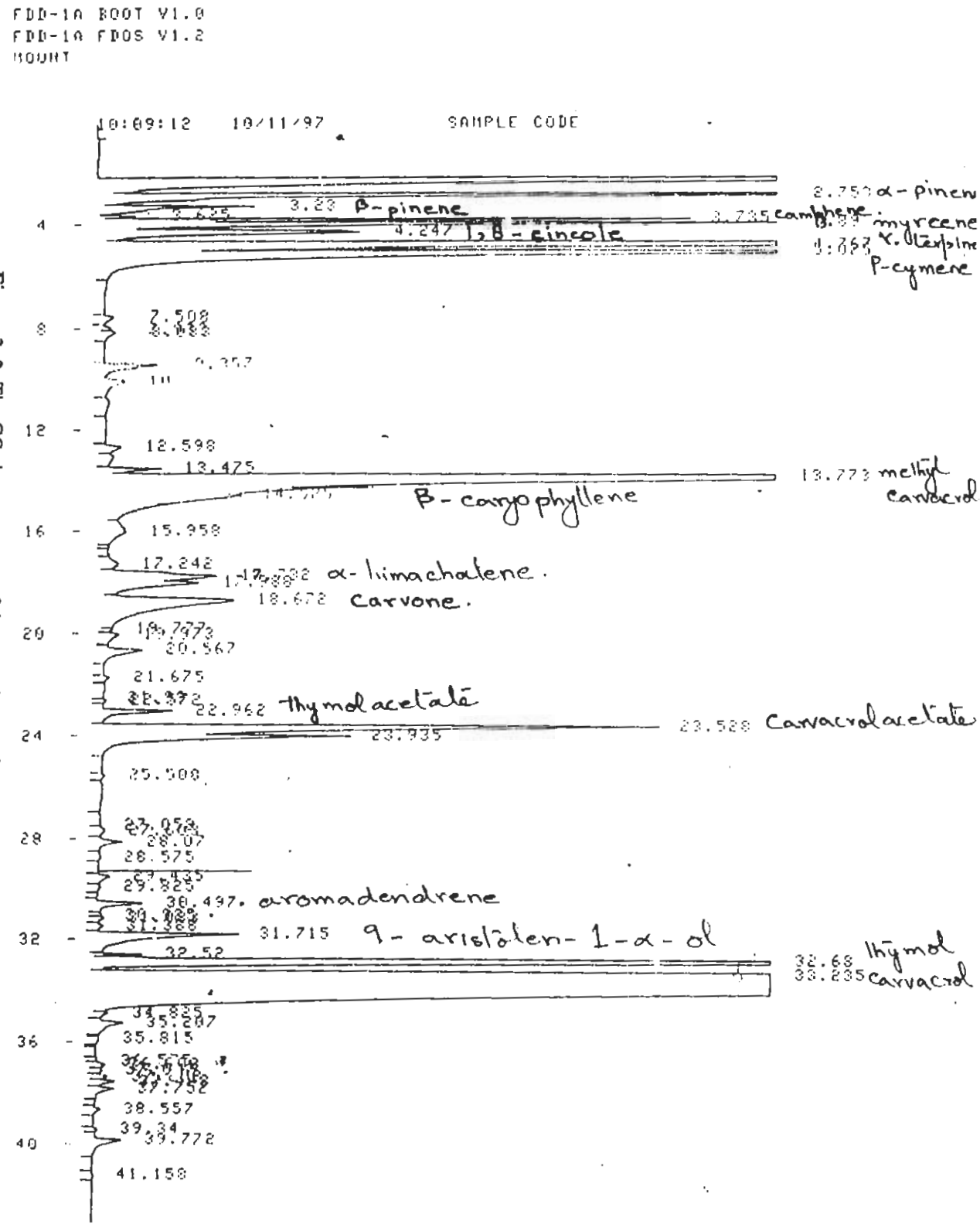


Figure 3.2: The GC chromatogram of the essential oil of *Zataria multiflora* Boiss on a Supelcowax™ 10

3.4.4 Calculation of the Retention Indices using Van den Dool and Kratz Formula

Since mass spectra of chemical constituents of the essential oils are not informative enough to distinguish between closely related compounds, it is advisable to support mass spectral data with GC retention indices. For this purpose Kovats retention index provides useful, reliable and in a specific column, relatively invariant retention data (150-152).

According to Kovats original definition (150, 151), the retention index is a 100-multiple of the carbon number of a hypothetical n-alkane that would display on a given column and under given conditions, the same t'_R (adjusted retention time) as the solute compound under investigation. Hence, in order to determine the retention index of solute i, it is necessary to measure, on a given column at constant conditions, the t'_R values for solute i and at least two reference *n*-alkane with carbon numbers z and z+n. The retention index I_i (eq. 3.1), is then calculated by the equation:

$$I_i = 100 \left[z + n \frac{\log (t_{R,i}/t'_{R,z})}{\log (t'_{R,z+n}/t'_{R,z})} \right]$$

eq. 3.1

The above formula is under constant temperature, so for a temperature programmed condition we have to use the modified Van den Dool and Kratz equation (eq. 3.2) (152, 153) in which C_n and C_{n+1} are the carbon

numbers of the standards which bracket the unknown peak in retention time, T_n is the retention time of the unknown, and S_n, S_{n+1} are retention times of the standards which bracket the retention time of the unknown.

$$RI = 100\ C_n + 100\ (C_{n+1} - C_n)\ \frac{(T_n - S_n)}{(S_{n+1} - S_n)}$$

eq. 3.2

For calculation of the RI of each compound, a sample of *n*-alkanes from C_8 to C_{18} (20 mg of each hydrocarbon in 1 ml ether) have been analysed by GC in the same chromatographic condition as for the essential oil's analysis. The results of the calculation are presented in Table-3.1.

3.4.5 GC/MS Analysis of the Essential Oil

For GC/MS experiments a Hewlett-Pakard 5890 gas chromatograph was combined with a JEOL, JMS-HX 110 mass spectrometer operating in EI mode at 70 eV. The condition for GC were column, Supelcowax™ 10 (25 m X 0.32 mm and 0.25 µm film thickness), carrier gas was helium and temperature programme was 60°C to 250°C at 5°C/min. Flow rate of carrier gas was 1 ml/min.

3.4.6 Qualitative Identification of the Essential Oil of *Zataria multiflora* Using GC, and GC/MS.

After calculation of retention indices (RI) for each peak in the GC chromatogram of the oil, the RI obtained for the constituents of the essential oil were compared with the literature values (128-131), and those compounds which have close values to our results were selected

for preliminary identification. Comparison of the mass spectrum of each of our components obtained by GC/MS, with those reported for the selected compounds (132-134) permitted us to identify the constituents of the oil.

3.4.7 Fractionation of the Oil

After preliminary identification of the component by GC and MS for confirming the results, the oil was fractionated and subjected to ^{13}C-NMR spectroscopy experiments.

The oil (1 g) was resolved into non polar and polar fractions by column chromatography, using silica gel (70-230 mesh, 40 g). The column was eluted by hexane to recover hydrocarbons and then ethyl acetate to obtain oxygenated compounds. Each fraction after concentration in vacuum was subjected to ^{13}C-NMR spectroscopy experiments. The non polar fraction contained monoterpene hydrocarbons and methyl carvacrol and the polar fraction consisted of almost pure carvacrol.

3.4.8 Identification by ^{13}C-NMR Spectroscopy

Identification by GC/MS was further confirmed by comparison of the ^{13}C-NMR (in C_6D_6) spectra of the mixture with those recorded for the pure authentic compounds in the literature (74, 135). The chemical shift value obtained for the compounds in the oil were in good agreement with the reported ones.

3.4.9 Identification by Co-injection

Carvacrol, thymol and their acetates which have the same mass spectral data were further identified by co-injection in the same chromatographic conditions of the oil analysis. The results of the analysis are given bellow:

	$t_{R, STD}$ (min.)	$t_{R, Oil}$ (min.)
Thymol	32.23	32.68
Thymol OAc	22.98	22.96
Carvacrol	33.95	33.23
Carvacrol OAc	23.29	23.52

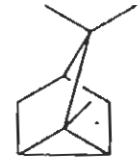
The analysis was done on the supelcowax 10 column with the same condition of chromatography. $t_{R, STD}$=retention time of authentic sample, $t_{R, Oil}$=retention time of the oil's components.

Table-3.1: The retention time of *n*-alkanes, and constituents of the oil, and calculated relative retention indices (RI).

Compound	t_R (min.)	RI	*n*-Alkane	t_R (min.)
tricyclene	13.89	919	C_8	10.44
α-thujene	14.22	927	C_9	13.16
α-pinene	14.68	939	C_{10}	16.98
camphene	15.70	966	C_{11}	21.68
β-pinene	16.09	976	C_{12}	26.49
myrcene	16.73	993	C_{13}	31.39
p-cymene	17.69	1015	C_{14}	36.06
1,8-cineole	17.94	1020	C_{15}	40.54
γ-terpinene	19.43	1052	C_{16}	44.77
terpinolene	20.44	1073	C_{17}	48.85
terpinen-4-ol	24.67	1163	C_{18}	52.78
α-terpineol	25.26	1174		
carvone	26.91	1208		
methyl carvacrol	28.06	1232		
carvacrol	32.58	1325		
thymol acetate	33.52	1345		
carvacrol acetate	34.78	1372		
β-caryophyllene	36.96	1420		
aromadendrene	37.93	1441		
α-himachalene	38.69	1458		
valencene	40.25	1493		
9-aristolen-1-α-ol	43.56	1571		
guaiol	44.46	1592		

3.4.10 Spectral Data of the Chemical Constituents of the Essential Oil of *Zataria multiflora*.

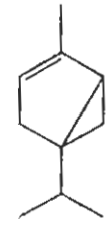
3.4.10.1 Tricyclene



EIMS *m/z* (rel.int.): 136 $[M]^+$ (20), 121 (25), 105 (10), 93 (100), 91 (20), 79 (20), 77 (15), 65 (5), 55 (6), 41 (20).

$C_{10}H_{16}$

3.4.10.2. α-Thujene

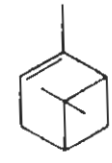


EIMS *m/z* (rel.int.): 136 $[M]^+$ (10), 121 (5), 105 (3), 93 (100), 91 (30), 79 (10), 77 (30), 65 (5), 41 (10).

^{13}C-NMR (in C_6D_6, 75.4 MHz): δ 16.0 (q), 19.9 (q), 20.2 (q), 21.8 (t), 30.8 (d), 33.2 (d), 34.0 (s), 36.9 (t), 121.4 (d), 145.9 (s).

$C_{10}H_{16}$

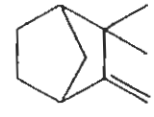
3.4.10.3. α-pinene



EIMS *m/z* (rel.int.): 136 $[M]^+$ (25), 121 (30), 107 (10), 105 (10), 93 (100), 79 (18), 77 (20), 67 (7), 53 (5).

$C_{10}H_{16}$

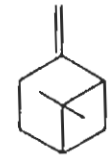
3.4.10.4. Camphene



EIMS *m/z* (rel.int.): 136 $[M]^+$ (30), 121 (95), 107 (37), 93 (100), 79 (32), 77 (20), 67 (27), 55 (10).

$C_{10}H_{16}$

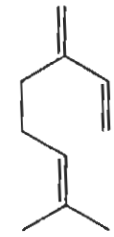
3.4.10.5. β-pinene



EIMS *m/z* (rel.int.): 136 $[M]^+$ (18), 121 (20), 107 (7), 93 (100), 79 (35), 77 (25), 69 (50), 55 (7).

$C_{10}H_{16}$

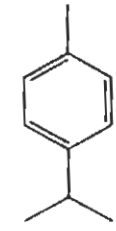
3.4.10.6. Myrcene



$C_{10}H_{16}$

EIMS *m/z* (rel.int.): 136 $[M]^+$ (12), 121 (10), 107 (5), 93 (100), 79 (15), 77 (15), 69 (75), 53 (10), 41 (20).

3.4.10.7. *p*-Cymene

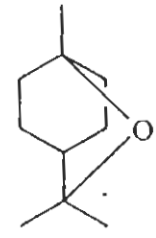


$C_{10}H_{14}$

EIMS (rel.int.): 134 $[M]^+$ (90), 119 (100), 105 (17), 103 (25), 91 (100), 77 (35), 65 (35), 63 (17), 58 (16), 51 (19).

^{13}C-NMR (in C_6D_6, 75.4 MHz): δ 20.9 (q), 24.2 (q x 2), 34.1 (d), 126.5 (d x 2), 129.3 (d x 2), 135.2 (s), 145.9 (s).

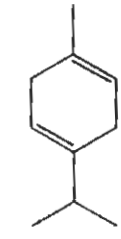
3.4.10.8. 1,8-Cineol



$C_{10}H_{18}O$

EIMS (rel.int.): 154 $[M]^+$ (70), 139 (45), 121 (40), 111 (50), 108 (62), 96 (40), 93 (100), 84 (65), 81 (85), 71 (75), 69 (65), 55 (60), 43 (60).

3.4.10.9. γ-Terpinene



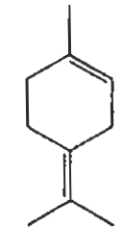
$C_{10}H_{16}$

EIMS (rel.int.): 136 $[M]^+$ (50), 121 (80), 107 (15), 105 (20), 93 (100), 91 (65), 79 (40), 77 (60), 65 (15), 53 (18), 43 (18).

^{13}C-NMR (in C_6D_6, 75.4 MHz): δ 21.5 (q x 2), 23.0 (q), 28.0 (t), 32.0 (t), 34.9 (d), 116.5 (d), 119.4 (d), 131.0 (s), 140.7 (s).

3.4.10.10. Terpinolene

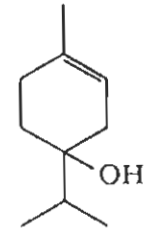
EIMS *m/z* (rel.int.): 136 $[M]^+$ (80), 121 (70), 107 (22), 105 (20), 93 (100), 91 (35), 79 (35), 77 (30), 67 (15), 53 (25), 44 (15).



$C_{10}H_{16}$

3.4.10.11. Terpinene-4-ol

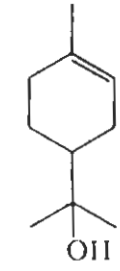
EIMS *m/z* (rel.int.): 154 $[M]^+$ (25), 136 (30), 121 (25), 111 (55), 93 (78), 91 (20), 86 (27), 77 (22), 71 (100), 69 (25), 55 (18), 53 (15), 43 (20).



$C_{10}H_{18}O$

3.4.10.12. α-Terpineol

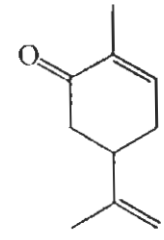
EIMS *m/z* (rel.int.): 154 $[M]^+$ (0), 136 $[M-H_2O]^+$ (35), 121 (50), 107 (15), 105 (10), 93 (80), 81 (35), 68 (65), 59 (100), 55 (25), 53 (30), 43 (15).



$C_{10}H_{18}O$

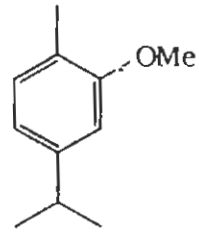
3.4.10.13 Carvone

EIMS (rel.int.): 150 $[M]^+$ (12), 135 (7), 121 (5), 108 (35), 93 (32), 82 (100), 79 (12), 67 (10), 58 (20), 54 (60).



$C_{10}H_{14}O$

3.4.10.14 Methyl Carvacrol

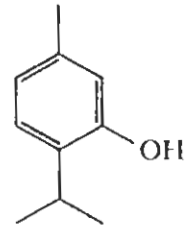


$C_{11}H_{16}O$

EIMS *m/z* (rel.int): 164 $[M]^+$ (98), 149 (99), 134 (58), 119 (61), 105 (30), 91 (100), 77 (50), 65 (25), 51 (20).

^{13}C-NMR (in C_6D_6, 75.4 MHz): δ 15.5 (q), 24.3 (q x 2), 34.6 (d), 54.8 (q), 108.7 (d), 118.4 (d), 124.2 (s), 130.9 (d), 147.9 (s), 158.3 (s).

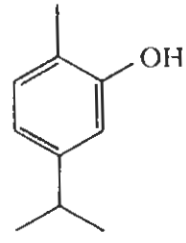
3.4.10.15 Thymol



$C_{10}H_{14}O$

EIMS *m/z* (rel.int.): 150 $[M]^+$ (98), 135 (100), 117 (22), 115 (25), 107 (10), 91 (45), 77 (22), 68 (12), 65 (16), 51 (17).

3.4.10.16 Carvacrol



$C_{10}H_{14}O$

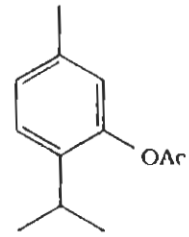
EIMS *m/z* (rel.int.): 150 $[M]^+$ (90), 135 (100), 117 (65), 107 (80), 91 (98), 77 (98), 65 (48), 51 (50).

^{1}H-NMR (in C_6D_6, 300 MHz): δ 1.16 (d, J=6.9, 3H-8, 3H-9), 1.26 (s, 3H-10), 2.72 (sep, J=6.9 Hz, H-7), 6.65 (dd, J=1.7, 7.6 Hz, H-5), 6.76 (d, J=1.7, H-3), 6.98 (dd, J=0.7, 7.6 Hz, H-6).

^{13}C-NMR (in C_6D_6, 75.4 MHz): δ 15.6 (q), 23.9, (q x 2), 33.8 (d), 113.1 (d), 118.0 (d), 121.9 (s), 130.8 (d), 148.0 (s), 155.2 (s).

3.4.10.17 Thymol acetate

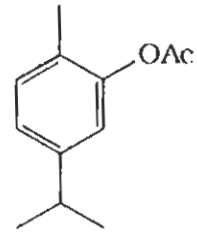
EIMS *m/z* (rel.int.): 192 $[M]^+$ (17), 150 (45), 135 (100), 115 (5), 105 (5), 91 (8), 77 (5), 65 (3), 43 (4).



$C_{12}H_{16}O_2$

3.4.10.18 Carvacrol acetate

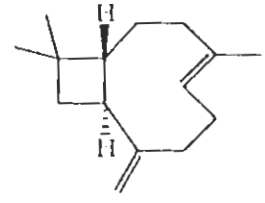
EIMS *m/z* (rel.int.): 192 $[M]^+$ (90), 150 (90), 135 (100), 121 (15), 105 (20), 91 (55), 77 (30), 65 (15), 51 (12), 43 (30).



$C_{12}H_{16}O_2$

3.4.10.19 β-Caryophyllene

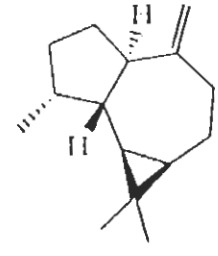
EIMS *m/z* (rel.int.): 204 $[M]^+$ (15), 189 $[M-Me]^+$ (20), 175 (10), 161 (35), 148 (25), 133 (70), 120 (35), 107 (40), 105 (45), 93 (95), 91 (70), 79 (75), 69 (100), 55 (70), 41 (40).



$C_{15}H_{24}$

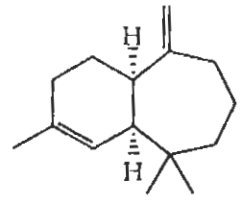
3.4.10.20 Aromadendrene

EIMS *m/z* (rel.int.): 204 $[M]^+$ (90), 189 (50), 175 (12), 161 (100), 147 (50), 133 (35), 119 (60), 105 (70), 93 (65), 81 (50), 69 (45), 55 (60), 44 (35).



$C_{15}H_{24}$

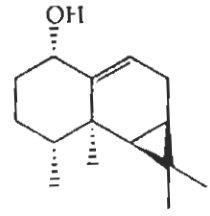
3.4.10.21 α-Himachalene



$C_{15}H_{24}$

EIMS *m/z* (rel.int.): 204 $[M]^+$ (40), 189 (30), 175 (8), 161 (50), 147 (15), 121(65), 107 (50), 105 (45), 93 (100), 79 (40), 59 (65).

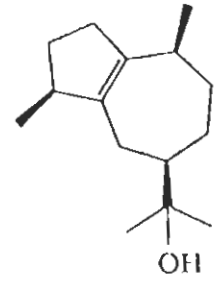
3.4.10.22 9-Aristolene-1α-ol



$C_{15}H_{24}O$

EIMS *m/z* (rel.int.): 220 $[M]^+$ (5), 202 $[M-H_2O]^+$ (60), 205 $[M-Me]^+$ (25), 187 $[M-C_3H_7]+$ (35), 159 (100), 145 (40), 55 (25).

3.4.10.23 Guaiol



$C_{15}H_{26}O$

EIMS *m/z* (rel.int.): 222 $[M]^+$ (0), 204 $[M-H_2O]^+$ (90), 189 (50), 175 (12), 161 (100), 147 (50), 133 (38), 119 (64), 107 (58), 105 (70), 93 (65), 91 (58), 81 (50), 69 (45), 55 (60), 44 (38).

Appendix A: X-ray Crystallographic Data for decipinone (79)

Table A-1: Crystal data and summary of intensity measurements, data collection and structure refinement of decipinone (**79**).

Formula	$C_{35}H_{45}O_{12}$
Color and shape	Colorless/plate-like
Mol. wt.	654.69
Space group	$P2_12_12$ (#18)
Crystal system	Orthorhombic
Temperature	295 (1) K
Lattice parameters	
a	15.574 (3) Å
b	19.620 (4) Å
c	11.314 (2) Å
Cell volume (V)	3457.1 (11) $Å^3$
Formula units/unit cell (Z)	4
Density calc.	1.258 g cm^{-3}
μ (Cukα)	0.79 mm^{-1}
Diffractometer	Enraf-Nonius CAD-4
Radiation, graphite monochromator	Cukα (λ=1.5418 Å)
Crystal dimensions	0.60 x 0.50 x 0.40 mm
Reflections measured	unique: 5134
2θ max	60.0°
2θ range	25 (20.0-30.0°)
Range of h, k, l	0 to 17, -21 to 22, 0 to 12
No. observations (I>2.0 σ (I))	4252
Structure solution	Direct methods (SAPI91)
Refinement	Full-matrix least-squares
Residuals: R; wR	0.076, 0.202

Table A:-2 Final fractional coordinates and equivalent isotropic displacement parameters (Å^2) for decipinone (**79**).

Atom	X	Y	Z	U_{eq}	Occupancy
O1	0.7864 (2)	0.4232 (2)	0.1609 (3)	0.0664 (8)	
O2	0.8795 (4)	0.5171 (3)	0.1442 (7)	0.145 (2)	
O3	0.8352 (2)	0.3122 (2)	-0.0156 (3)	0.0680 (9)	
O4	0.9574 (3)	0.3679 (3)	0.0265 (5)	0.1030 (14)	
O5	0.8616 (2)	0.1878 (2)	-0.1209 (3)	0.0707 (9)	
O6	0.7780 (3)	0.1240 (2)	-0.2310 (3)	0.0972 (13)	
O7	0.9781 (2)	0.2238 (2)	0.1896 (3)	0.0644 (8)	
O8	1.1040 (3)	0.2336 (3)	0.0974 (4)	0.1060 (15)	
O9	0.7025 (2)	0.1099 (2)	0.3264 (3)	0.0670 (8)	
O10	0.7501 (3)	0.1791 (2)	0.4706 (3)	0.0839 (11)	
O11	0.6015 (2)	0.2256 (2)	0.3231 (4)	0.0843 (11)	
O12	0.8026 (2)	0.2920 (2)	0.3406 (3)	0.0640 (8)	
C1	0.6687 (4)	0.3574 (3)	0.3180 (5)	0.0703 (13)	
C2	0.6400 (3)	0.3919 (3)	0.2070 (5)	0.0710 (14)	
C3	0.7156 (3)	0.3810 (2)	0.1226 (5)	0.0639 (12)	
C4	0.7384 (3)	0.3065 (2)	0.1449 (4)	0.0571 (11)	
C5	0.8272 (3)	0.2847 (2)	0.1029 (4)	0.0542 (10)	
C6	0.8418 (3)	0.2059 (2)	0.0917 (4)	0.0555 (10)	
C7	0.9412 (3)	0.1973 (3)	0.0819 (4)	0.0623 (12)	
C8	0.9654 (3)	0.1238 (3)	0.0651 (6)	0.078 (2)	
C9	0.9175 (4)	0.0749 (3)	0.1107 (5)	0.081 (2)	
C10	0.8705 (4)	0.0514 (3)	0.2985 (6)	0.093 (2)	
C11	0.8411 (3)	0.0867 (2)	0.1891 (5)	0.0668 (12)	
C12	0.8127 (3)	0.1625 (2)	0.2010 (4)	0.0559 (10)	
C13	0.7151 (3)	0.1623 (2)	0.2346 (4)	0.0586 (11)	
C14	0.6759 (3)	0.2281 (2)	0.2873 (4)	0.0605 (11)	
C15	0.7233 (3)	0.2953 (2)	0.2782 (4)	0.0570 (11)	
C16	0.6145 (4)	0.4658 (3)	0.2261 (7)	0.098 (2)	
C17	0.8012 (3)	0.1783 (3)	-0.0231 (4)	0.0640 (12)	
C18	0.9219 (6)	0.0833 (4)	0.3815 (7)	0.121 (3)	
C19	0.8436 (7)	-0.0201 (4)	0.3104 (9)	0.148 (4)	
C20	0.8065 (4)	0.4779 (3)	0.0928 (6)	0.0754 (15)	
C21	0.7706 (4)	0.4916 (3)	0.0043 (5)	0.0676 (13)	

C22	0.9047 (5)	0.3515 (3)	-0.0426 (6)	0.084 (2)	
C23	0.8898 (7)	0.3685 (5)	-0.1791 (8)	0.102 (2)	0.632 (9)
C24	0.8226 (7)	0.3412 (6)	-0.2447 (11)	0.134 (4)	0.632 (9)
C25	0.8138 (9)	0.3579 (8)	-0.3634 (11)	0.230 (9)	0.632 (9)
C26	0.8721 (12)	0.4018 (10)	-0.4166 (9)	0.293 (14)	0.632 (9)
C27	0.9393 (10)	0.4291 (8)	-0.3511 (13)	0.243 (11)	0.632 (9)
C28	0.9482 (7)	0.4124 (6)	-0.2323 (13)	0.197 (8)	0.632 (9)
C23A	0.9066 (6)	0.3772 (2)	-0.1499 (6)	0.102 (2)	0.368 (9)
C24A	0.8830 (5)	0.3381 (2)	-0.2470 (5)	0.134 (4)	0.368 (9)
C25A	0.8824 (7)	0.3668 (3)	-0.3593 (6)	0.230 (9)	0.368 (9)
C26A	0.9055 (8)	0.4347 (3)	-0.3745 (8)	0.293 (14)	0.368 (9)
C27A	0.9292 (9)	0.4738 (3)	-0.2774 (9)	0.243 (11)	0.368 (9)
C28A	0.9297 (8)	0.4451 (2)	-0.1651 (8)	0.197 (8)	0.368 (9)
C29	0.8427 (2)	0.1578 (2)	-0.2208 (3)	0.0658 (12)	
C30	0.9088 (3)	0.1660 (3)	-0.3096 (3)	0.097 (2)	
C31	1.0599 (3)	0.2405 (3)	0.1774 (3)	0.088 (2)	
C32	1.0896 (3)	0.2737 (3)	0.2989 (4)	0.148 (4)	
C33	0.6524 (3)	0.1386 (3)	0.1385 (4)	0.0669 (12)	
C34	0.7195 (4)	0.1243 (3)	0.4375 (5)	0.0702 (13)	
C35	0.6956 (5)	0.0686 (3)	0.5198 (5)	0.100 (2)	

$U_{eq} = (1/3)\Sigma_i\Sigma_j U^{ij} a_i^* a_j^* a_i . a_j$

Table A-3: Molecular dimensions for decliptinone (79).

A) Bond distances (Å)

O1 C20	1.356 (7)	O1 C3	1.445 (6)
O2 C20	1.492 (8)	O3 C22	1.364 (7)
O3 C5	1.451 (5)	O4 C22	1.178 (8)
O5 C29	1.309 (4	O5 C17	1.464 (6)
O6 C29	1.212 (5)	O7 C31	1.323 (5)
O7 C7	1.444 (6)	O8 C31	1.44 (6)
O9 C34	1.316 (6)	O9 C13	1.474 (5)
O10 C34	1.235 (6)	O11 C14	1.227 (6)
O12 C15	1.423 (5)	C1 C2	1.495 (8)
C1 C15	1.553 (7)	C2 C16	1.519 (8)
C2 C3	1.532 (7)	C3 C4	1.524 (6)
C4 C5	1.525 (6)	C4 C15	1.541 (7)
C5 C6	1.568 (6)	C6 C17	1.542 (6)
C6 C7	1.560 (6)	C6 C12	1.568 (6)
C7 C8	1.504 (7)	C8 C9	1.320 (8)
C9 C11	1.503 (7)	C10 C18	1.383 (10)
C10 C19	1.470 (10)	C10 C11	1.490 (8)
C11 C12	1.557 (6)	C12 C13	1.568 (6)
C13 C33	1.533 (6)	C13 C14	1.548 (7)
C14 C15	1.516 (7)	C20 C21	1.178 (8)
C22 C23A	1.315 (9)	C22 C23	1.597 (11)
C29 C30	1.45 (2)	C31 C32	1.59 (2)
C34 C35	1.482 (8)		

B) Bond angles (°)

C20 01 C3	117.3 (4)	C22 03 C5	119.0 (4)
C29 05 C17	116.8 (3)	C31 07 C7	112.6 (3)
C34 09 C13	119.9 (4)	C2 C1 C15	106.0 (4)
C1 C2 C16	113.1 (5)	C1 C2 C3	103.3 (4)
C16 C2 C3	115.0 (5)	01 C3 C4	108.8 (4)
01 C3 C2	108.7 (4)	C4 C3 C2	102.1 (4)
C5 C4 C3	115.4 (4)	C5 C4 C15	113.7 (4)
C3 C4 C15	105.3 (4)	03 C5 C4	105.1 (4)
03 C5 C6	106.2 (3)	C4 C5 C6	115.7 (4)
C17 C6 C7	108.0 (4)	C17 C6 C5	110.8 (4)
C7 C6 C5	104.8 (4)	C17 C6 C12	110.9 (4)
C7 C6 C12	106.5 (4)	C5 C6 C12	115.4 (4)
07 C7 C8	110.6 (4)	07 C7 C6	107.3 (4)
C8 C7 C6	111.2 (4)	C9 C8 C7	120.4 (5)
C8 C9 C11	124.5 (5)	C18 C10 C19	122.3 (7)
C18 C10 C11	122.1 (6)	C19 C10 C11	115.6 (7)
C10 C11 C9	100.1 (5)	C10C11C12	117.3 (4)
C9 C11 C12	115.0 (4)	C11 C12 C13	107.0 (4)
C11C12 C6	111.6 (4)	C13 C12 C6	118.3 (4)
09 C13 C33	101.8 (4)	09 C13 C14	105.0 (3)
C33 C13 C14	105.9 (4)	09 C13 C12	107.6 (3)
C33 C13 C12	116.5 (4)	C14 C13 C12	118.3 (4)
011 C14 C15	121.1 (4)	011 C14 C13	117.8 (4)
C15 C14 C13	120.5 (4)	012 C15 C14	110.5 (4)
012 C15 C4	111.1 (4)	C14 C15 C4	105.4 (4)
012 C15 C1	111.5 (4)	C14 C15 C1	113.3 (4)
C4 C15 C1	104.8 (4)	05 C17 C6	109.1 (4)
C21 C20 01	123.7 (5)	C21 C20 02	125.1 (6)
01 C20 02	111.3 (5)	04 C22 C23A	119.4 (6)
04 C22 03	124.0 (6)	C23A C22 03	116.2 (7)
04 C22 C23	133.3 (6)	03 C22 C23	102.6 (7)
C24 C23 C22	123.1 (8)	C28 C23 C22	116.9 (8)
06 C29 05	121.0 (4)	06 C29 C30	125.9 (3)
05 C29 C30	112.9 (2)	08 C31 07	129.1 (4)
08 C31 C32	123.9 (3)	07 C31 C32	107.0 (2)
010 C34 09	123.6 (5)	010 C34 C35	123.3 (5)
09 C34 C35	113.1 (5)		

C) Selected torsion angles (°)

C15 C1 C2 C3	-34.0 (5)	C1 C2 C3 C4	43.4 (5)
C2 C3 C4 C15	-36.1 (5)	C3 C4 C15 C1	15.6 (5)
C2 C1 C15 C4	11.5 (5)	C15 C4 C5 C6	74.9 (5)
C4 C5 C6 C12	-48.3 (5)	C5 C6 C12 C13	63.1 (5)
C6 C12 C13 C14	-70.5 (5)	C12 C13 C14 C15	15.1 (6)
C13 C14 C15 C4	57.1 (5)	C5 C4 C15 C14	-97.3 (4)
C12 C6 C7 C8	59.8 (5)	C6 C7 C8 C9	-30.5 (7)
C7 C8 C9 C11	-5.0 (9)	C8 C9 C11 C12	8.1 (8)
C9 C11 C12 C6	24.5 (6)	C7 C6 C12 C11	-56.4 (5)

Appendix B: X-ray Crystallographic Data for decipinol ester A (88)

Table B-1: Crystal data and summary of intensity measurements, data collection and structure refinement of decipinol ester A (88).

Formula	$C_{33}H_{40}O_{11}$
Color and Shape	Colorless/plate-like
Mol. wt.	620.78
Space group	$P2_12_12_1$ (#19)
Crystal system	Orthorhombic
Temperature	22.0°C
Lattice parameters	
a	8.893 (4) Å
b	17.509 (2) Å
c	20.276 (4) Å
Cell volume (V)	3157 (1) $Å^3$
Formula units/unit cell (Z)	4
Density calc.	1.29 g cm^{-3}
μ (Cukα)	8.05 cm^{-1}
Diffractometer	Enraf-Nonius CAD-4
Radiation, graphite monochromator	Cukα (λ=1.54178 Å)
Crystal dimensions	0.50 x 0.48 x 0.22 mm
Decay of standard reflections	2%
Reflections measured	unique: 3246
2θ max	135.8°
Cell determination (2θ range)	25 (40.0-50.0°)
No. observations (I>2.0 σ (I))	1266
Structure solution	Direct methods (SIR92)
Refinement	Full-matrix least-squares
Residuals: R; wR	0.0671; 0.1386

Table B-2 Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$ x 103) for decipinol ester A (88). U (eq) is defined as one third of the trace of the orthogonalized Uij tensor.

Atom	X	Y	Z	U_{eq}
O(1)	10247 (8)	200 (3)	7679 (3)	76 (2)
O(2)	9062 (13)	-467 (5)	6886 (5)	144 (4)
O(3)	7532 (7)	-253 (3)	8886 (3)	72 (2)
O(4)	8812 (9)	-1216 (4)	8404 (4)	98 (2)
O(5)	8113 (8)	1830 (3)	9504 (4)	90 (2)
O(6)	9069 (7)	-881 (3)	9969 (3)	77 (2)
O(7)	7257 (12)	-1587 (5)	10413 (5)	139 (4)
O(8)	12190 (7)	1751 (4)	9786 (4)	84 (2)
O(9)	9814 (9)	2713 (3)	9275 (4)	100 (2)
O(10)	11365 (7)	1250 (3)	8529 (3)	73 (2)
O(11)	12401 (9)	2436 (4)	8456 (4)	106 (3)
C(1)	9350 (13)	2023 (5)	8038 (5)	93 (4)
C(2)	9020 (14)	1453 (6)	7483 (6)	93 (3)
C(3)	8903 (12)	651 (5)	7800 (5)	75 (3)
C(4)	8815 (11)	835 (5)	8534 (5)	71 (3)
C(5)	8914 (10)	185 (5)	9010 (4)	66 (2)
C(6)	8875 (10)	467 (4)	9736 (5)	63 (2)
C(7)	8288 (12)	-192 (5)	10190 (5)	79 (3)
C(8)	8533 (15)	-43 (6)	10897 (6)	100 (4)
C(9)	9865 (16)	238 (6)	11090 (6)	103 (4)
C(10)	11083 (12)	439 (5)	10613 (5)	77 (3)
C(11)	10492 (10)	737 (5)	9932 (5)	65 (3)
C(12)	10627 (11)	1612 (5)	9903 (5)	72 (3)
C(13)	9669 (13)	1908 (5)	9322 (6)	81 (3)
C(14)	9827 (12)	1525 (5)	8623 (5)	71 (3)
C(15)	10082 (16)	1507 (6)	6913 (6)	127 (5)

C(16)	10184 (17)	-347 (6)	7215 (6)	99 (4)
C(17)	11577 (15)	-789 (6)	7178 (6)	122 (5)
C(18)	7650 (15)	-949 (5)	8589 (5)	80 (3)
C(19)	6172 (7)	-1297 (4)	8522 (4)	77 (3)
C(20)	6081 (10)	-1996 (4)	8200 (4)	150 (6)
C(21)	4707 (12)	-2371 (4)	8152 (4)	192 (8)
C(22)	3426 (9)	-2047 (5)	8428 (5)	123 (5)
C(23)	3517 (8)	-1348 (5)	8750 (4)	111 (4)
C(24)	4890 (9)	-973 (3)	8797 (4)	98 (4)
C(25)	7697 (12)	1116 (4)	9812 (5)	85 (3)
C(26)	8410 (17)	-1505 (6)	10118 (6)	99 (4)
C(27)	9350 (15)	-2205 (5)	9856 (6)	124 (5)
C(28)	12239 (13)	-204 (7)	10555 (5)	88 (3)
C(29)	13684 (13)	54 (7)	10260 (6)	127 (5)
C(30)	12064 (17)	-899 (6)	10778 (6)	131 (5)
C(31)	10165 (12)	2036 (5)	10532 (5)	97 (4)
C(32)	12496 (13)	1753 (6)	8452 (5)	84 (3)
C(33)	13962 (12)	1319 (7)	8357 (6)	111 (4)

Table B-3: Molecular dimensions for decipinol ester A (**88**).

Bond lengths [Å]	
O(1)-C(16)	1.343 (11)
O(1)-C(3)	1.454 (11)
O(2)-C(16)	1.219 (13)
O(3)-C(18)	1.363 (10)
O(3)-C(5)	1.471 (10)
O(4)-C(18)	1.195 (12)
O(5)-C(13)	1.439 (11)
O(5)-C(25)	1.446 (10)
O(6)-C(26)	1.352 (12)
O(6)-C(7)	1.462 (10)
O(7)-C(26)	1.188 (13)
O(8)-C(12)	1.431 (11)
O(9)-C(13)	1.418 (9)
O(10)-C(32)	1.346 (11)
O(10)-C(14)	1.463 (11)
O(11)-C(32)	1.199 (10)
C(1)-C(2)	1.532 (13)
C(1)-C(14)	1.532 (12)
C(2)-C(15)	1.495 (15)
C(2)-C(3)	1.548 (12)
C(3)-C(4)	1.524 (13)
C(4)-C(5)	1.495 (11)
C(4)-C(14)	1.519 (11)
C(5)-C(6)	1.552 (12)
C(6)-C(25)	1.554 (11)
C(6)-C(11)	1.565 (12)

C(6)-C(7)	1.566 (11)
C(7)-C(8)	1.474 (14)
C(8)-C(9)	1.340 (15)
C(9)-C(10)	1.494 (14)
C(10)-C(28)	1.529 (13)
C(10)-C(11)	1.568 (13)
C(11)-C(12)	1.538 (11)
C(12)-C(31)	1.532 (12)
C(12)-C(13)	1.543 (13)
C(13)-C(14)	1.573 (13)
C(16)-C(17)	1.462 (16)
C(18)-C(19)	1.455 (13)
C(19)-C(20)	1.3900
C(19)-C(24)	1.3900
C(20)-C(21)	1.3900
C(21)-C(22)	1.3900
C(22)-C(23)	1.3900
C(23)-C(24)	1.3900
C(26)-C(27)	1.509 (14)
C(28)-C(30)	1.308 (13)
C(28)-C(29)	1.487 (14)
C(32)-C(33)	1.521 (13)

Bond angles [°]

C(16)-O(1)-C(3)	118.1 (9)
C(18)-O(3)-C(5)	118.5 (8)
C(13)-O(5)-C(25)	116.0 (7)
C(26)-O(6)-C(7)	116.4 (8)
C(32)-O(10)-C(14)	119.9 (7)

C(2)-C(1)-C(14)	104.6 (7)
C(15)-C(2)-C(1)	113.9 (10)
C(15)-C(2)-C(3)	114.8 (9)
C(1)-C(2)-C(3)	107.4 (8)
O(1)-C(3)-C(4)	108.8 (8)
O(1)-C(3)-C(2)	111.6 (8)
C(4)-C(3)-C(2)	102.5 (7)
C(5)-C(4)-C(14)	119.6 (8)
C(5)-C(4)-C(3)	117.9 (7)
C(14)-C(4)-C(3)	104.8 (8)
O(3)-C(5)-C(4)	103.7 (7)
O(3)-C(5)-C(6)	108.0 (7)
C(4)-C(5)-C(6)	111.7 (7)
C(5)-C(6)-C(25)	110.0 (8)
C(5)-C(6)-C(11)	108.4 (7)
C(25)-C(6)-C(11)	111.9 (7)
C(5)-C(6)-C(7)	109.3 (7)
C(25)-C(6)-C(7)	104.8 (7)
C(11)-C(6)-C(7)	112.3 (8)
O(6)-C(7)-C(8)	111.9 (9)
O(6)-C(7)-C(6)	105.6 (7)
C(8)-C(7)-C(6)	113.1 (8)
C(9)-C(8)-C(7)	118.7 (11)
C(8)-C(9)-C(10)	122.5 (11)
C(9)-C(10)-C(28)	111.3 (9)
C(9)-C(10)-C(11)	113.9 (9)
C(28)-C(10)-C(11)	113.8 (8)
C(12)-C(11)-C(6)	111.3 (7)
C(12)-C(11)-C(10)	109.8 (8)

C(6)-C(11)-C(10)	115.5 (8)
O(8)-C(12)-C(31)	108.4 (8)
O(8)-C(12)-C(11)	104.6 (7)
C(31)-C(12)-C(11)	115.5 (9)
O(8)-C(12)-C(13)	110.6 (9)
C(31)-C(12)-C(13)	109.0 (8)
C(11)-C(12)-C(13)	108.7 (7)
O(9)-C(13)-O(5)	101.5 (8)
O(9)-C(13)-C(12)	109.5 (8)
O(5)-C(13)-C(12)	107.6 (9)
O(9)-C(13)-C(14)	110.8 (9)
O(5)-C(13)-C(14)	106.0 (9)
C(12)-C(13)-C(14)	119.7 (8)
O(10)-C(14)-C(4)	106.0 (7)
O(10)-C(14)-C(1)	110.2 (8)
C(4)-C(14)-C(1)	101.3 (8)
O(10)-C(14)-C(13)	110.0 (9)
C(4)-C(14)-C(13)	113.2 (8)
C(1)-C(14)-C(13)	115.5 (8)
O(2)-C(16)-O(1)	122.7 (13)
O(2)-C(16)-C(17)	125.1 (11)
O(1)-C(16)-C(17)	112.2 (11)
O(4)-C(18)-O(3)	123.7 (10)
O(4)-C(18)-C(19)	126.0 (9)
O(3)-C(18)-C(19)	110.3 (10)
C(20)-C(19)-C(24)	120.0
C(20)-C(19)-C(18)	117.8 (7)
C(24)-C(19)-C(18)	122.2 (7)
C(19)-C(20)-C(21)	120.0

C(22)-C(21)-C(20)	120.0
C(21)-C(22)-C(23)	120.0
C(24)-C(23)-C(22)	120.0
C(23)-C(24)-C(19)	120.0
O(5)-C(25)-C(6)	114.6 (8)
O(7)-C(26)-O(6)	121.9 (12)
O(7)-C(26)-C(27)	128.3 (12)
O(6)-C(26)-C(27)	109.8 (11)
C(30)-C(28)-C(29)	121.6 (12)
C(30)-C(28)-C(10)	125.4 (12)
C(29)-C(28)-C(10)	112.9 (10)
O(11)-C(32)-O(10)	126.8 (11)
O(11)-C(32)-C(33)	124.0 (12)
O(10)-C(32)-C(33)	109.1 (9)

4. REFERENCES

1. Evans, F.J. and Taylor, S.E., (1983), in: *Progress in the Chemistry of Organic Natural Products*, (Herz, W.; Grisebach, H.; and Kirby, G.W., eds), Volume **44**, pp.1-99, Springer-Verlag, New York.

2. Singla, A.K. and Pathak, K., *Fitoterapia*, 1990, **LXI**, 483-516.

3. Wu, T.S.; Lin, Y.M.; Haruna, M.; Pan, D.J.; Shingu, T.; Chen, Y.P.; Hsu, H.Y.; Nakano, T., and Lee, K.H., *J.Nat.Prod.*, 1991, **54**, 823-829.

4. Öksüz, S.; Gürek, F.; Gil, R.R.; Pengsuparp, T.; Pezzuto, J.M. and Cordell, G.A., *Phytochemistry*, 1995, **38**, 1457-1462.

5. Rechinger, K.H. (1964), in: *Flora Iranica*; (Rechinger, K.H. and Schiman-Czeika, H., eds.) No.6, pp.33-34, Akademische Druck and Verlagsanstalt, Graz, Austria.

6. Radcliffe-Smith, A., (1986), in: *Flora of Pakistan*, (Nasir, E. and Ali, S.I, eds.) No. 172, p.88, Shamim Printing Press, Karachi, Pakistan.

7. Smith-Kielland, I.; Dornish, J.M.; Malterud, K.E.; Hvistendahl, G.; Romming, Chr.; Bockman, O.C.; Kolsaker, P.; Stenstrom, Y and Nordal, A., *Planta Med.*, 1966, **62**, 322-325.

8. Satti, N.K.; Suri, O.P.; Thaper, R.K. and Kachroo, P.L., *Phytochemistry*, 1988, **27**, 1530-1531.

9. Singh, A. and Agarwal, R.A., *Acta Hydrochim.Hydrobiol.*, 1992, **20**, 262-264. (*Chem. Abst.* 1993, **119**, 22791m).

10. Saraf, S. and Dixit, V.K. *Indian Drugs*, 1994, **31**, 28-31.

11. Costa, E.; Aguado, J.; Ovejero, G. and Canizares, P., An. Quim., 1991, **87**, 71-75 (*Chem.Abst.* 1991, **115**, 95845j).

12. Borghi, D.; Baumer, L.; Ballabio, M.; Arlandini, E.; Perellino, N.C.; Minghetti, A. and Vincieri, F.F., *J.Nat.Prod.* 1991, **54**, 1503-1508.

13. Speroni, E.; Coletti, B.; Minghetti, A.; Perellino, N.C.; Guicciardi, A.; and Vincieri, F.F., *Planta Med.*, 1991, **57**, 531-535.

14. Öksüz, S.; Gil, R.R.; Chai, H.; Pezzuto, J.M., Cordell, G.A. and Ulubelen, A., *Planta Med.*, 1994, **60**, 594-596.

15. Gminski, R. and Hecker, E., *Planta Med.*, 1991, **57**, A50.

16. Falsone, G.; Cateni, F.; Katusian, F.; Wagner, H.; Seligmann, O.; Pellizer, G. and Asaro, F., *Planta Med.*, 1993, **59**, A617.

17. Seip, E.H. and Hecker, E., *Phytochemistry*, 1983, **22**, 1791-1795.

18. Gundidza, M.; Mavi, S., and Chinyanganya, *Planta Med.*, 1993, **59**, A704.

19. Abo, K.A., *Fitoterapia*, 1990, **XLI**, 462-463.

20. Hohmann, J.; Vasas, A.; Günther, G.; Mathe, I.; Evanics, F.; Dombi, G., and Jerkovich, G., *J.Nat.Prod.* 1997, **60**, 331-335.

21. Manners, G.D., and Wong, R.Y., *J.Chem.Soc.PerkinTrans.I*, 1985, 2075-2081.

22. Onwukaeme, N.D., and Rowan, M.G., *Phytochemistry*, 1992, **31**, 3479-3482.

23. Zhang, H. and Ding, Y., *Zhiwu Ziyuan Yu Huanjing*, 1992, **1**, 6-9 (*Chem.Abst.* 1993, **118**, 35926p).

24. Wju, L. *Chem.Abst.* 1994, **120**, 331115x.

25. Lin, L.J., and Kinghorn, A.D., *Phytochemistry*, 1983, **22**, 2795-2799.

26. Lanhers, M.-C; Fleurentin, J.; Dorfman, P.; Mortier, F. and Pelt, J.-M., *Planta Med.*, 1991, **57**, 225-231.

27. Galvez, J.; Zarzuelo, A.; Crespo, M.E.; Lorente, M.D.; Ocete, M.A and Jimenez, J., *Planta Med.*, 1993, **59**, 333-336.

28. Pan, D.-J.; Hu, C. -Q.; Chang, J.-J.; Lee T. T.-Y.; Chen, Y.-P.; Hsu, H.-Y.; Mcphail, D.R.; Mcphail, A.T. and Lee, K.-H., *Phytochemistry*, 1991, **30**, 1018-1020.

29. Shi, J.-G; Jia, Z.-J. and Cui, Y.-X., *J.Nat.Prod.*, 1995, **58**, 51-56.

30. Cruz, C.M.; Kasper, P.; Cataldo, A.; Zamith, H.P.S. and Paumgartten, F.J.R.; Braz., *J.Med.Biol.Res.*, 1996, **29**, 1519-1523 (*Chem.Abst.* 1997, **126**, 27921h).

31. Zani, C.L.; Marston, A.; Hamburger, M. and Hostettmann, K., *Phytochemistry*, 1993, **34**, 89-95.

32. Yamamoto, Y., *Techno-Cosmos*, 1994, **5**, 19-24 (*Chem.Abst.* 1995, **122**, 316805v)

33. Hecker, E. and Sosath, S. *CLB Chem.Labor.Betr.*, 1991, **42**, 121-2. (*Chem.Abst.* 1991, **114**, 222871w).

34. Vogel, R.; Pascual-Villalobos, M.J. and Roebbelen, G., *Angew.Bot.* 1993, **67**, 31-41.

35. Peng, X.and Wei, K., *Chem.Abst.*, 1995, **122**, 125979v.

36. Fakunle, C.O.; Connolly, J.D., and Rycroft, D.S., *Fitoterapia*, 1992, **LXIII**, 329-332.

37. Satyanarayana, V.; Krupadanam, G.L.D. and Srimannarayana, G., *Indian.J.Chem.*, 1991, **30B**, 989-990.

38. Satyanarayana, V.; Krupadanam, G.L.D. and Srimannarayana, G., *Fitoterapia*, 1992, **LXII**, 82-83.

39. Ferreira, M.J.U.; Lobo, A. M. and Wyler, H., *Fitoterapia*, 1993, **LXIV**, 85-87.

40. Singla, A.K., and Pathak, K., *Fitoterapia*, 1991, LXII, 453-454.

41. Awad, N.E.; Abou Zeid, A.H. and El-Missiry, M.M., *Fitoterapia*, **LXIV**,1993, 553.

42. Ferreira, M.J.U.; Lobo, A. M.; Nascimento, J.M. and Wyler, H., *Planta Med.*, 1994, **60**, 581.

43. Fatope, M.O.; Zeng, L.; Ohayaga, J.E.; Shi, G., and Mclaughlin, J.L., *J.Med.Chem.*, 1996, **39**, 1005-1008.

44. Fakunle, C.O.; Okogun, J.I.; Ekong, D.E.; Connolly, J.D. and Rycroft, D.S., *J.Chem.Soc. Perkin Trans.1*, 1990, 727-730.

45. Morgenstern, T.; Bittner, M.; Silva, M.; Aqueveque, P. and Jakupovic, J., *Phytochemistry*, 1996, **41**, 1149-1153.

46. Wu, D.; Sorg, B. and Hecker, E., *J.Nat.Prod.*, 1995, **58**, 408-413

47. Mbwambo, Z.H.; Lee, S.K.; Mshiu, E.N.; Pezzuto, J.M. and Kinghorn, A.D., *J.Nat.Prod.*, 1996, **59**, 1051-1055.

48. Schall, V.T.; Vasconellos, M.C.; Valent, G.U.; Sato, M.I.Z.; Furlan, E.V. and Sanchez, P.S., *Braz.J.Med.Biol.Res.*, 1991, **24**, 573-582. (*Chem.Abst.*, 1991, **115**, 247558h).

49. Sow, D.; Sall, M.D.; Depeyre, D.; Isambert, A. and Kayembe, S.M., *Chem.Abst.*, 1993, **118**, 172425p.

50. Dev, S. and Misra, R. (1986), in: CRC Handbook of Terpenoids, Diterpenoids (Dev, S. ed.), Vol. IV, pp 7-63, CRC Press, Inc. Boca Raton, Florida.

51. Ruzicka, L., *Experientia*, 1953, IX, 357-396.

52. Ruzicka, L.; *Balaš, Fr.* and *Vilim, Fr., Helv.Chim.Acta*, 1924, **7**, 458-471.

53. Karrer, P.; Morf, R. and Schöpp, K., *Helv.Chim.Acta*, 1931, **14**, 1036-1040 and 1431-1436.

54. Rentzea, M.; Hecker, E., and Lotter, H., *Tetrahedron Lett.*, 1982, **23**, 1781-1784.

55. Dewick, P.M., *Nat.Prod.Reports*, 1997, **14**, 111-144.

56. Lemmich, E., *Phytochemistry*, 1979, **18**, 1195-1197.

57. Teresa, J.D.P.; Caballero, E.; Caballero, C.; Medarde, M.; Barrero, A. F. and Grande, M., *Tetrahedron Lett.*, 1978, **37**, 3491-3494.

58. Dauben, W.G.; Thiessen, W.E.; and Resnick P.R., *J.Am.Chem. Soc.*, 1962, **84**, 2015-2016.

59. Kobayashi, H. and Akiyoshi, S., *Bull.Chem.Soc.Jpn.*, 1962, **35**, 1044-1045.

60. Herin, M.; Colin, M. and Fursen, B., *Bull.Soc.Chim.Belg.*, 1976, **85**, 801.

61. Nabeta, K.; Ishikawa, T., and Okuyama, H., *J.Chem.Soc.Perkin Trans.1*, 1995, **24**, 3111-3115.

62. Wenkert, E., *Chem.Ind.*, 1995, 282.

63. Adolf, W. and Hecker, E., *Isr.J.Chem.*, 1977, **16**, 75.

64. Rentzea, M., and Hecker, E., *Tetrahedron Lett.*, 1982, **23**, 1785-1788.

65. Jeske, F.; Jakupovic, J. and Berendsohn, W., *Phytochemistry*, 1995, **40**, 1743-1750.

66. Appendino, G.; Jakupovic, S.; Tron, G.C.; Jakupovic, J.; Milon, V., and Ballero, M., *J.Nat.Prod.*, 1993, **61**, 749-756.

67. Jakupovic, J.; Morgenstern, T.; Marco, J.A. and Berendsohn, W., *Phytochemistry*, 1998, **47**, 1611-1619.

68. Jakupovic, J.; Morgenstern, T.; Bittner, M. and Silva, M., *Phytochemistry*, 1998, **47**, 1601-1609.

69. Jakupovic, J.; Jeske, F ; Morgenstern, T.; Tsichritzis, F.; Marco, J.A. and Berendsohn, W., *Phytochemistry*, 1998, **47** 1583-1600.

70. Guenther, E., (1948), *The Essential Oils*, D. Van Nostrand Company Inc., New York.

71. *British Pharmacopoeia*, (1988), Vol II, pp. A132-A135, Her Majesty's Stationery Office, London.

72. Husain, A.; Virmani, O.P.; Sharma, A.; Kumar, A., and Misra, L.N., (1988), *Major Essential Oil-Bearing Plants of India*, Central Institute of Medicinal and Aromatic Plants, Lucknow, India.

73. Pushpangadan, P., (1993) in: *Newer Trends in Essential Oils and Flavours*, (Dhar, K.L.; Thappa, R.K. and Agarwal, S.G., eds) pp. 308-321, Mc Graw-Hill, New Delhi.

74. Formacek, V., and Kubeczka, K.-H., (1982) in: *Essential Oils Analysis by Capillary Gas Chromatography and Carbon-13 NMR spectroscopy*, John Wiley & Sons.

75. *British Pharmacopoeia*, (1988), Volume II, pp. A137-A138, Her Majesty's Stationery Office, London.

76. Jarvis, A.P. and Morgan, E.D., *Phytochem.Anal.*, 1997, **8**, 217-222.

77. Vilegas, J.H.Y.; Lancas, F.M., and Vilegas, W., *Flav.Frag.J.*, 1994, **4**, 39-43.

78. Masada, Y., (1976), *Analysis of Essential Oils by Gas Chromatography and Mass Spectrometry*, John Wiley & Sons, Inc., New York.

79. Weyerstahl, P.; Marschall-Weyerstahl., H.; Weirauch, M.; Meier, N.; Manteuffel, E.; Leimner, J., and Scholz, S. (1986), in: *Progress in Essential Oil Research*, (Brunke, E.-J. ed.), pp. 177-195, Walter de Gruyter Co. Berlin, New York.

80. Herres, W.; Kubeczka, K.H. and Schultze, W. (1986), in:: *Progress in Essential Oil Research*, (Brunke, E.-J. ed.), pp. 507-528, Walter de Gruyter Co. Berlin, New York.

81. Öksüz, S.; Gürek, F.; Yang, S.-W.; Lin, L.-Z.; Cordell, G.A.; Pezzuto, J.M.; Wagner, H., and Lotter, H., *Tetrahedron*, 1997, **53**, 3215-3222.

82. Yamamura, S.; Shizuri, Y.; Kosemura, S.; Ohtsuka, J.; Tayama, T.; Ohba, S.; Ito, M.; Saito, Y. and Terada, Y., *Phytochemistry*, 1989, **28**, 3421-3436.

83. Narayanan, P.; Röhrl, M.; Zechmeister, K.; Engel, D.W.; Hoppe, W.; Hecker, E. and Adolf, W., *Tetrahedron Lett.*, 1971, **18**, 1325-1328.

84. Morita, H.; Gonda, A.; Wei, L.; Yamamura, Y.; Takeya, K. and Itokawa, H., *J.Nat.Prod.*, 1997, **60**, 390-392.

85. Marco, J.A.; Sanz-Cervera, J.F. and Yuste, A., *Phytochemistry*, 1997, **45**, 563-570.

86. Adolf, W.; Hecker, E.; Balmain, A.; Lhomme, M.F.; Nakatani, Y.; Ourisson, G.; Ponsinet, G.; Pryce, R.J.; Santhanakrishnan, T.S.; Matyukhina, L.G., and Saltikova, I.A., *Tetrahedron Lett.*, 1970, **26**, 2241-2244.

87. Green, R.W., and Tong, H.K., *J.Amer.Chem.Soc.*, 1956, **78**, 4896-4900.

88. Dommisse, R.; Freyne, E.; Esmans, E.; Lepoivre, J. and Alderweireldt, F., *Heterocycles*, 1981, **16**, 1893-1897.

89. Khan, A.Q. and Malik, A., *J.Nat.Prod.*, 1990, **53**, 728-731.

90. Chen, H. and Jia, Z.J., *Ind.J.Chem.*, 1996, **35B**, 1308-1310.

91. Öksüz, S.; Gürek, F.; Lin, L.-Z.; Gil, R.R.; Pezzuto, J.M. and Cordell, G.A., *Phytochemistry*, 1996, **42**, 473-478.

92. Yang, L.; Shi, Y.-P.; Jia, Z.-J.; Saleh, S. and Lahham, J., *J.Nat.Prod.*, 1995, **58**, 1883-1888.

93. Buckingham, J., (1994), in: *Dictionary of Natural Products*, : Vol.5, p. 5258, Chapman & Hall.

94. Aizawan, K.; Yoshida, S. and Takahashi, N., *Org.Mass spectrum.*, 1974, **9**, 470-479.

95. Thompson, M.J.; Dutky, S.R.; Patterson, G.W. and Gooden, E.L., *Phytochemistry*, 1972, **11**, 1781-1790.

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

97. Tavares, O.S.; Duarte, N.D.; Cunha, S.O.; Ascenso, J.R. and Ferreira, M.J.U., *Fitoterapia*, 1995, **LXVI**, 551-552.

98. Kikuchi, T.; Kadota, S., and Tsubono, K., *Chem.Pharm.Bull.* 1986, **34**, 2479-2486.

99. Itoh, T.; Ishii, T.; Tamura, T., and Matsumoto, T., *Phytochemistry*, 1978, **17**, 971-977.

100. Rauter, A.P.; Calindro, J.M.; Silva J.M. and Quintal, R., *Planta Med.*, 1990, **56**, 546.

101. Teresa, J.D.P.; Urones, J.G.; Marcos, I.S.; Basabe, P.; Cuadrado, M.J.S., and Moro, R.F., *Phytochemistry*, 1987, **26**, 1767-1776.

102. Djerassi, G., and McCrindle, R., *J.Chem.Soc.*, 1962, 4034-4039.

103. Öksüz, S.; Shieh, H.-L; Pezzuto, J.M.; Özhatay, N. and Cordell, G.A., *Planta Med.*, 1993, **59**, 472-473.

104. Aplin, R.T., and Hornby, G.M., *J.Chem.Soc.* (B), 1966, 1078-1079.

105. Upadhyay, R.R.; Moinzadeh, F.; Bunakdari, A.; Sedehi, F., and Samin, R., *Pol.J.Chem.*, 1983, **57**, 1387-1388.

106. Wu, D.; Sorg, B., and Hecker, E., *J. Nat. Prod.*, 1995, **58**, 408-413.

107. Öksüz, S.; Gürek, F.; Qiu, S.X. and Cordell, G.A., *J.Nat.Prod.*, 1988, **61**, 1198-1201.

108. Gali, H.U.; Perchellet, E.M. and Perchellet, J.P., *Cancer Res.*, 1991, **51**, 2820-2825.

109. Ferreira J.; Coloma, L.; Fones, E.; Letelier, M.E.; Repetto, Y.; Morello, A., and Aldunate, J., *FEBS Lett.*, 1988, **234**, 285-488.

110. Sekiguchi, T., and Yoshida, K., *Chem.Abst.*, 1989, **111**, 87273y.

111. Weast, R.C.; Grasselli, J.G., (eds.) (1989) *CRC Hanbook of Data on Organic Compounds*, Vol.2, p.1146, Boca Raton, Florida.

112. Sasaki, S. (ed.) (1985), *Handbook of Proton-NMR Spectra and Data*, Academic Press, Tokyo.

113. Budzikiewicz, H.; Wilson, J.M., and Djerassi, G., *J.Am.Chem.Soc.*, 1963, **85**, 3688-3699.

114. Sholichin, M.; Yamasaki, K.; Kasai, R., and Tanaka, O., *Chem.Pharm.Bull.*, 1980, **28**, 1006-1008.

115. Das, S.C., *Chem.Ind.*, 1971, 1331-1332.

116. Herz, W.; Santhanam, P.S., and Wahlberg, I., *Phytochemistry*, 1972, **11**, 3061-3063.

117. Hui, W.H., and Lee, W.K., *J.Chem.Soc. (C)*, 1971, 1004-1006.

118. Doddrell, D.M.; Khong, P.W.; and Lewis, K.G., *Tetrahedron Lett.*, 1974, **27**, 2381-2384.

119. Ito, S.; Kodama, M.; Sunagawa, M; Oba, T., and Hikino, H., *Tetrahedron Lett.*, 1969, **34**, 2905-2908.

120. Ahmad, V.U., and Atta-ur-Rahman (1994), *Handbook of Natural Products Data*, Vol. 2, pp. 67,111,112, Elsevier, Netherland.

121. Seo, S.; Tomita, Y.; Tori, K., and Yoshimura, Y., *J.Am.Chem. Soc.*, 1978, **100**, 3331-3339.

122. Iribarren, A.M., and Pomilio, A.B., *Phytochemistry*, 1984, **23**, 2087-2088.

123. Hedge, I.C. (1990) in: *Flora of Pakistan*, (Ali, S.I. and Nasir, Y.L. eds.) No. 192, pp.252-254, BCCT Press, University of Karachi, Pakistan.

124. Gupta, G.S., and Gupta, N.L., *Phytochemistry*, 1972, **11**, 455-456.

125. Malik, M.S.; Sattar, A., and Khan, S.A., *Pakistan J.Sci.Ind.Res.*, 1987, **30**, 751-753.

126. Shafiee, A., and Javidnia, K., *Planta Med.*, 1997, **63**, 371-372.

127. Farooq, M.O., and Gupta, G.S., *Perfumery Essent.Oil Rec.*, 1954, **45**, 287.

128. Davies, N.W., *J.Chromatography*, 1990, **503**, 1-24.

129. Rustaiyan, A.; Komeilizadeh, H.; Sanei Shariatpanahi, M.; Jassbi, A.R., and Masoudi, S., *J.Essent.Oil.Res.*, 1998, **10**, 207-209.

130. Rustaiyan, A.; Masoudi, S., and Jassbi, A.R., *J.Essent.Oil Res.*, 1997, **9**, 599-600.

131. Rustaiyan, A.; Komeilizadeh, H.; Masoudi, S.; and Jassbi, A.R., *J. Essent.Oil Res.*, 1997, **9**, 713-714.

132. Ryhage, R., and Sydow, E.V., *Acta Chem.Scand.*, 1963, **17**, 2025-2035.

133. Sydow, E.V., *Acta Chem.Scand.*, 1964, **18**, 1099-1104.

134. Sydow, E.V., *Acta Chem.Scand.*, 1963, **17**, 2504-2512.

135. Bohlmann, F.; Zeisberg, R., and Klein, E., *Org.Mag.Reson.*,. 1975, **7**, 426-432.

136. Zdero, C.; Bohlmann, F.; Wasshausen, D.C., and Mungai, M.G., *Phytochemistry*, 1991, **30**, 4025-4028.

137. Rustaiyan, A.; Mosslemin-Kupaii, M.H.; Papastergion, F., and Jakupovic, J., *Phytochemistry*, 1995, **40**, 875-879.

138. Fan Hai-Fu, (1991), SAPI91, *Structure Analysis Programs with Intelligent Control*, Rigaku Corporation, Tokyo, Japan.

139. Beurskens, P.T.; Admiraal, G.; Beurskens, G.; Bosman, W.P.; de Gelder, R.; Israel, R., and Smits, J.M.M., (1994), DIRDIF94 *Technical Report of the Crystallography Laboratory*, University of Nijmegen, The Netherlands.

140. Cromer, D.T.; Waber, J.T., (1974), *International Tables for X-ray Crystallogrphy*, Vol. IV, Table 2.2A, The Kynoch Press, Birmingham, England.

141. Ibers, J.A., and Hamilton, W.C., *Acta Cryst.*, 1964, **17**, 781.

142. Creagh, D.C.; McAuley, W.J., (1992), *International Tables for Crystallography*, (A.J.C. Wilson, ed.) Vol. C, Table 4.2.6.8, pp.219-222, Kluwer Academic Publishers, Boston.

143. Creagh, D.C.; Hubbell, J.H., (1992), *International Tables for Crystallography* (A.J.C. Wilson, ed.) Vol.C., Table 4.2.4.3, pp.200-206, Kluwer Academic Publishers, Boston.

144. Molecular Structure Corporation (1994), *TEXSAN, Single Crystal Structure Analysis Software, MSC, 3200*, Research Forest Drive, The Woodlands, TX 77381, USA.

145. Sheldrick, G.M., (1993), *SHELXL93 Program for the Refinement of Crystal Structures*, University of Göttingen, Germany.

146. Flack, H.D., *Acta Cryst.*, 1983, **A39**, 876-881.

147. Altomare, A.; Cascarano, M.; Giacovazzo, C., and Guagliardi, A., (SIR92), *J.Appl.Cryst.*, 1993, **26**, 343.

148. Sheldrick, G.M. (1997), SHELXL97, *Program for Refinement of Crystal Structures*, University of Göttingen, Germany.

149. teXsan: *Crystal Structure analysis Package*, Molecular Structure Corporation (1985 and1992).

150. Novak, J., (1987), in: *Chromatograhphic Theory and Basic Principles* (Jönsson, J.A. ed.) p.114, Marcel Dekker, Inc. New York and Basel.

151. Kovats, E., *Helv.Chim.Acta*, 1958, **41**, 1915-1932.

152. Betty, K.R. and Karasek, F.W., *J.Chromatography*, 1978, **166**, 111-122.

153. Van den Dool, H. and Kratz, D.D., *J.Chromatography*, 1963, **11**, 463.

LIST OF PUBLICATIONS

1. A. Rustaiyan, M.M. Sabery and **A.R. Jassbi**, "Constituents of the Essential Oil of *Mentha mozaffariani* Jamzad.", *J.School of Pharmacy, Univ. of Tehran*, 1994, **4** (1,2), 13-25.

2. A. Nassir-Ahmadi, A. Rustaiyan and **A.R. Jassbi**, "Chemical Analysis of the Essential Oil of *Artemisia haussknechtii* Boiss.", *J. School of Pharmacy, Univ. of Tehran*, 1994, **4** (1,2), 39-49.

3. F. Mohammadi, **A.R. Jassbi** and A. Rustaiyan; "A Study of the Essential Oil of *Artemisia santolina* by GC-MS", *J. Sci. Islamic Azad Univ.*, 1992, **2**, 449-457.

4. A. Rustaiyan, A. Nassir Ahmadi, **A.R. Jassbi**, B. Abaszade Sarasti, R. Barikbin, R. Golmohammad, "Identificatioln and Determination of Dioctylphthalate in *Nepeta laxiflora*", *J. Sci. Islamic Azad Univ.*, 1992, **2**, 543-554.

5. A. Rustaiyan, A. Monfared, **A.R. Jassbi**, "*Nepeta denudata* as a New Source of 1,8-cineol", *J. Sci. Islamic Azad Univ.*, 1992, **2**, 623-632.

6. A. Rustaiyan, S.A. Sadjadi and **A.R. Jassbi**, "Constitutents of the Essential Oil of *Nepeta glomerulosa* Boiss.", *J. Sci. Islamic Azad Univ.*, 1993, **3**, 187-191.

7. V.U. Ahmad, **A.R. Jassbi** and M. Parvez, "Three New Diterpene Esters from *Euphorbia decipiens*", *Tetrahedron*, 1998, **54**(8), 1573-1584.

8. A. Rustaiyan, H. Komeilizadeh, S. Masoudi and **A.R. Jassbi**, "Constituents of the Essential Oil of *Salvia sahandica* Boiss. & Buhse.", *J.Essent.Oil Res.*, 1997, **9**, 713-714.

9. A. Rustaiyan, S. Masoudi and **A.R. Jassbi**, "Essential Oil of *Salvia hydrangea* DC. ex. Benth.", *J.Essent.Oil Res.*, 1997, **9**, 599-600.

10. A. Rustaiyan, H. Komeilizadeh, M. Sanei Shariatpannahi, **A.R. Jassbi** and S. Masoudi, "Comparative Study of the Essential Oils of Three *Achillea* Species from Iran", *J.Essent.Oil Res.*, 1998, **10**, 207-209.

11. V.U. Ahmad and **A.R. Jassbi**, "Two New Diterpene Esters from *Euphorbia decipiens*", *Phytochemistry*, 1998, **48** (7), 1217-1221.

12. V.U. Ahmad and **A.R. Jassbi**, Three Tricyclic Diterpenoids from *Euphorbia decipiens*, *Planta Med.*, 1998, **64**, 1-4.

13. **A.R. Jassbi**, V.U. Ahmad and R.B. Tareen, "Constituents of the Essential Oil of *Perovskia atriplicifolia* Benth.", *Flavour and Fragrance Journal*, (in press).

14. V.U. Ahmad, **A.R. Jassbi** and R.B., Tareen, "Constituents of the Essential Oil of *Zataria multiflora* Boiss. from Pakistan," *J.Essent.Oil Res.*, (in press).

15. V.U. Ahmad, **A.R. Jassbi** and M. Sanei Charlat Pannahi; "Analysis of the Essential Oil of *Echinophora sibthorpiana* Guss. by means of GC, GC-MS and ^{13}C-NMR Techniques", *J.Essent.Oil Res.*, 1999, **11**, 107-108.

16. V.U. Ahmad, **A.R. Jassbi**, F.N. Zafar and R.B. Tareen, "The Essential Oil of *Salvia cabulica* Benth.", *Plant.Med.*, 1999, **65**, 1-2.

17. F. Mojab, **A.R. Jassbi** and A. Rustaiyan; "Constituents of the Essential Oil of *Heracleum persicum* Desf.", *J. School Pharmacy, Univ. of Tehran* (accepted).

18. V.U. Ahmad, **A.R. Jassbi**; "Three New Diterpenoids from *Euphorbia teherarica*", *J.Nat.Prod.*, (sumitted).