PHYTOCHEMICAL INVESTIGATIONS OF TWO MEDICINAL PLANTS *EUPHORBIA CLARKEANA* AND *SERICOSTOMA PAUCIFLORUM*

THESIS SUBMITTED FOR
THE FULFILMENT OF THE DEGREE OF
DOCTOR OF PHILOSOPHY

BY

S. ABDUL MAJID AYATOLLAHI

H.E.J. RESEARCH INSTITUTE OF CHEMISTRY
UNIVERSITY OF KARACHI
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Dedicated to
Ar-Razi and Avicenna
The pioneers of Modern Sciences

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(S. ABDUL MAJID AYATOLLAHI)

CONTENTS

SUMMARY

This thesis presents the work in two parts. The part "A" describes the studies on the chemical constituents of Euphorbia clarkeana which have resulted in the isolation and characterization of a new triterpene and further five unreported compounds.

The part "B" deals with the chemical constituents of Sericostoma pauciflorum have resulted in the isolation and characterization of three new and three unreported compounds.

New Compound From *Euphorbia clarkeana*

Cycloclarkeanol (65)

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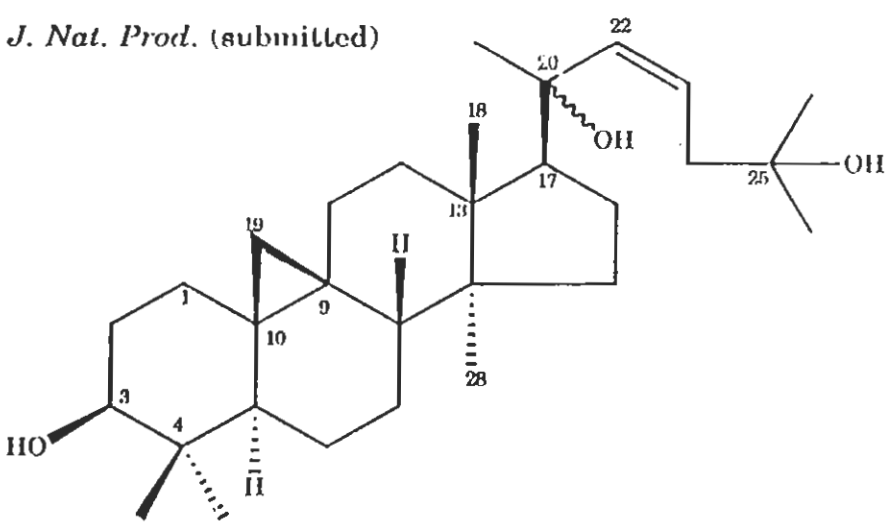

Unreported Compounds from *Euphorbia clarkeana*

1. Cycloartanol (**66**)

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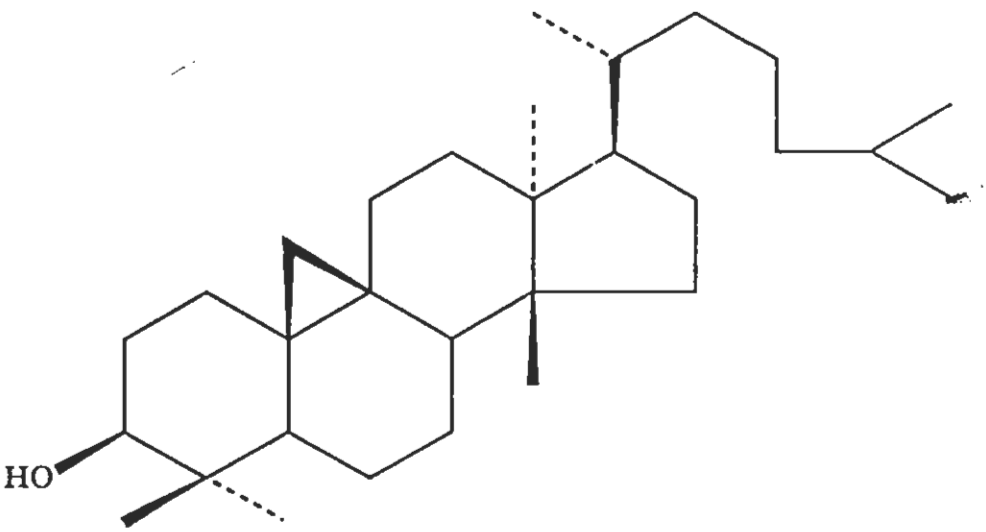

2. Cycloart-23-ene-3β,25-diol (**67**)

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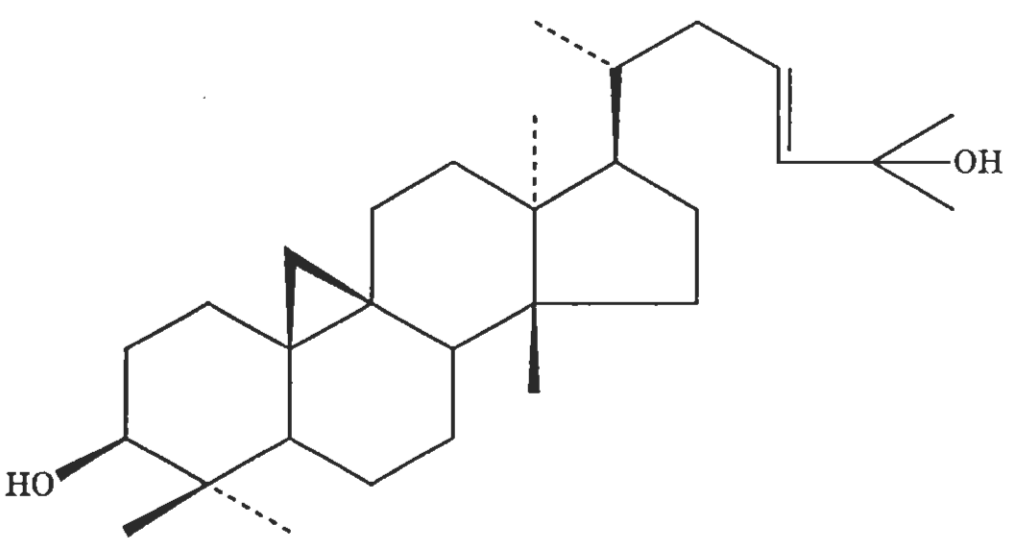

3. 20-Acetyl-ingenol-3-decadienoate (**68**)

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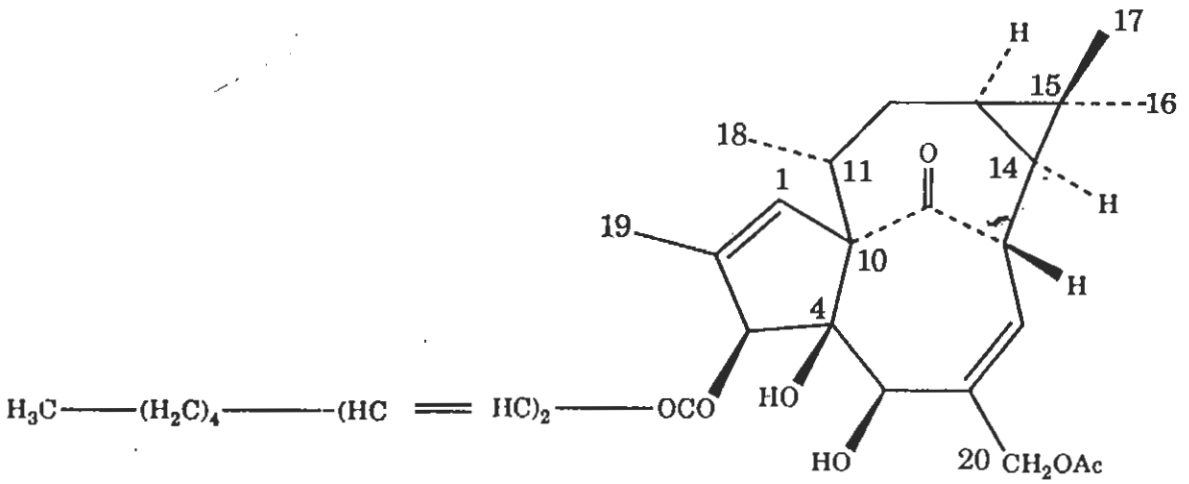

4. Lupeol (**69**)

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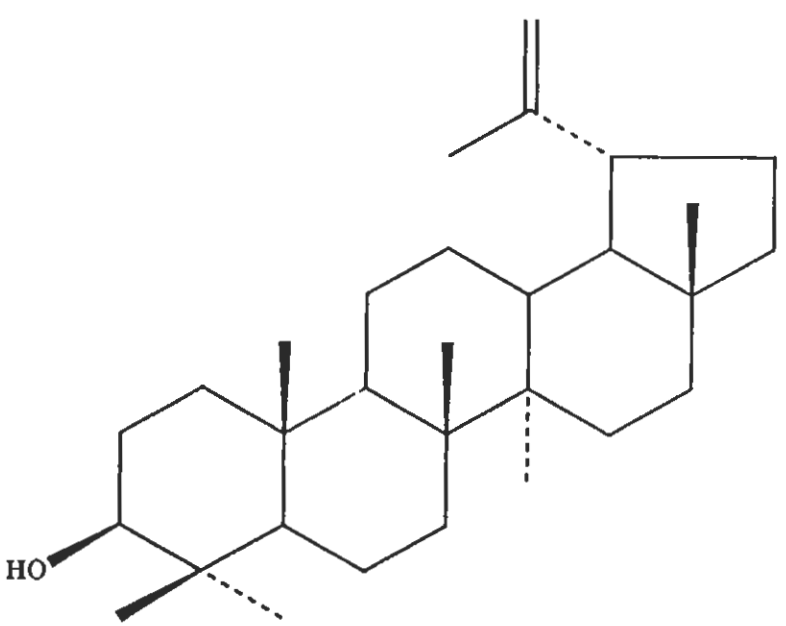

5. Taraxasterol (70)

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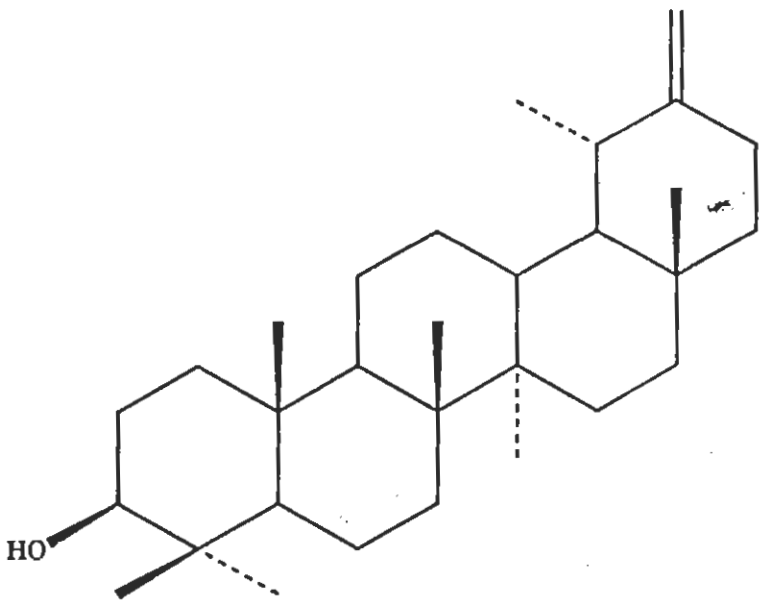
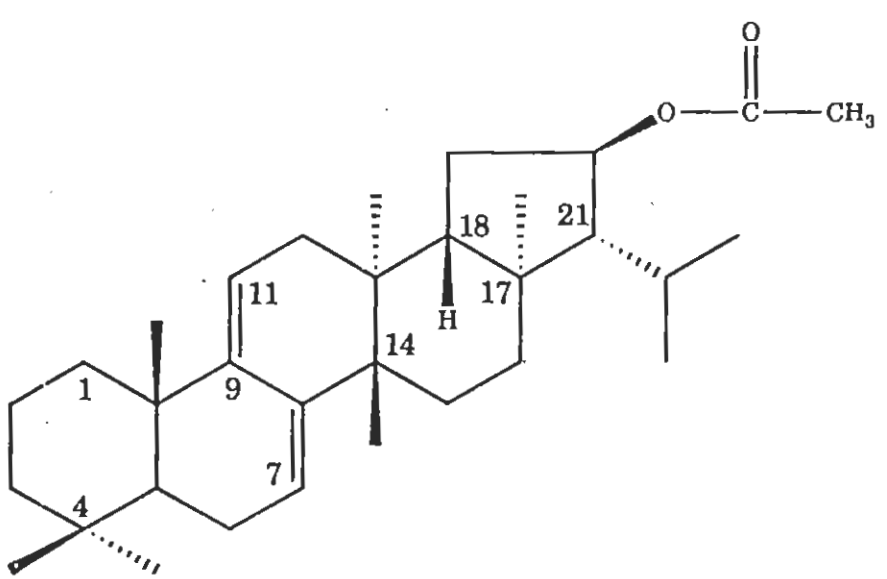


New Compounds from *Sericostoma pauciflorum*

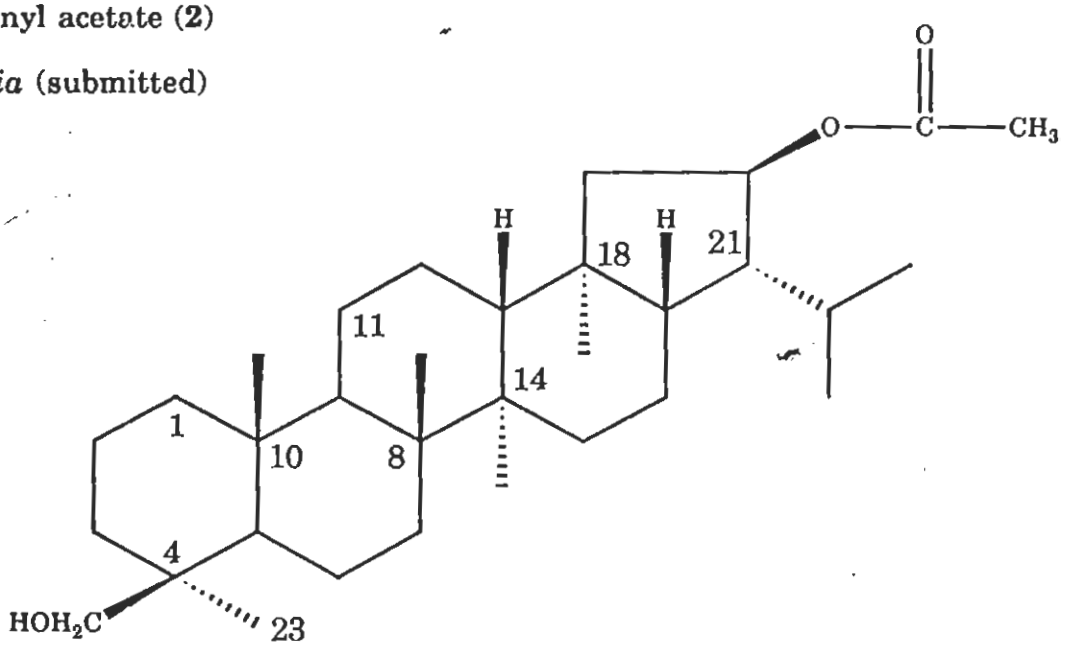
1. Sericostinyl acetate (1)

J. Nat Prod. **54**, 570 (1991)



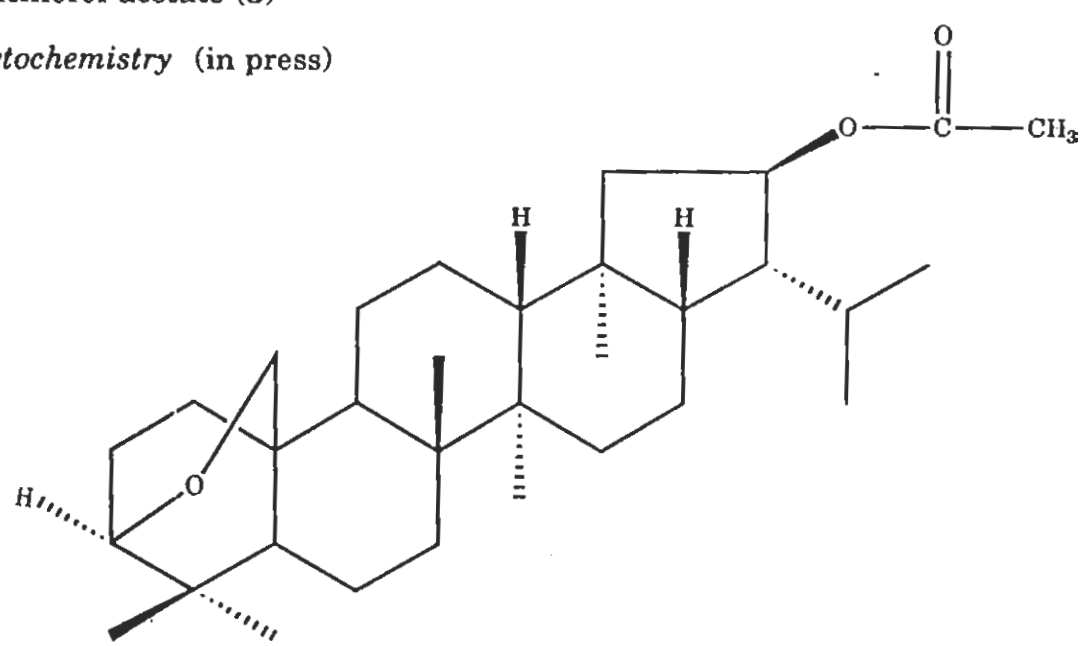
2. Pauciflorinyl acetate (2)

Fitoterapia (submitted)



3. Pauciflorol acetate (3)

Phytochemistry (in press)



Unreported Compounds from *Sericostoma pauciflorum*

4. α-Amyrin (**4**)

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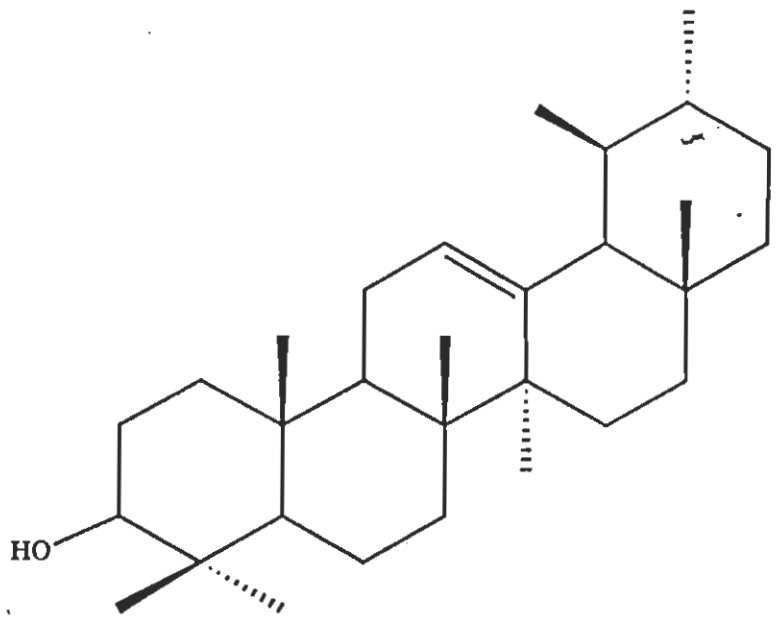

5. β-Amyrin (**5**)

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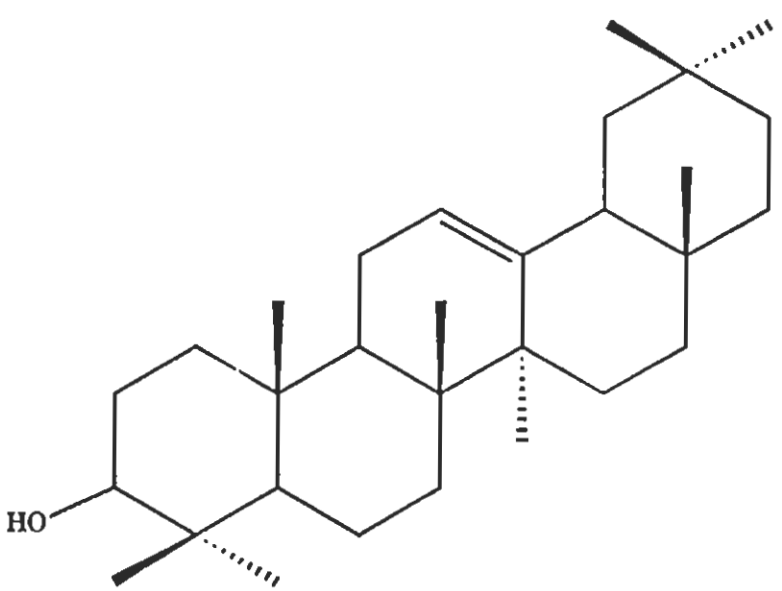
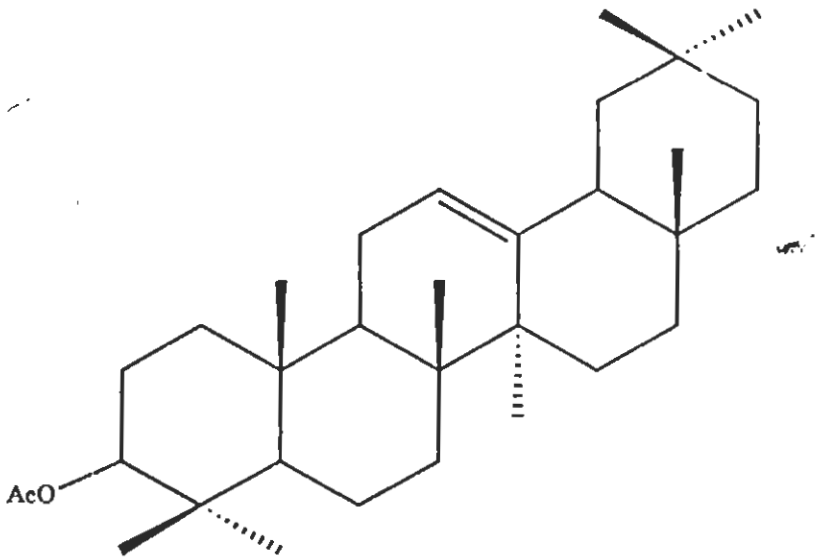


6. β-Amyrin acetate

J. Nat. Prod. **54**, 570 (1991)



7. β-Sitosterol

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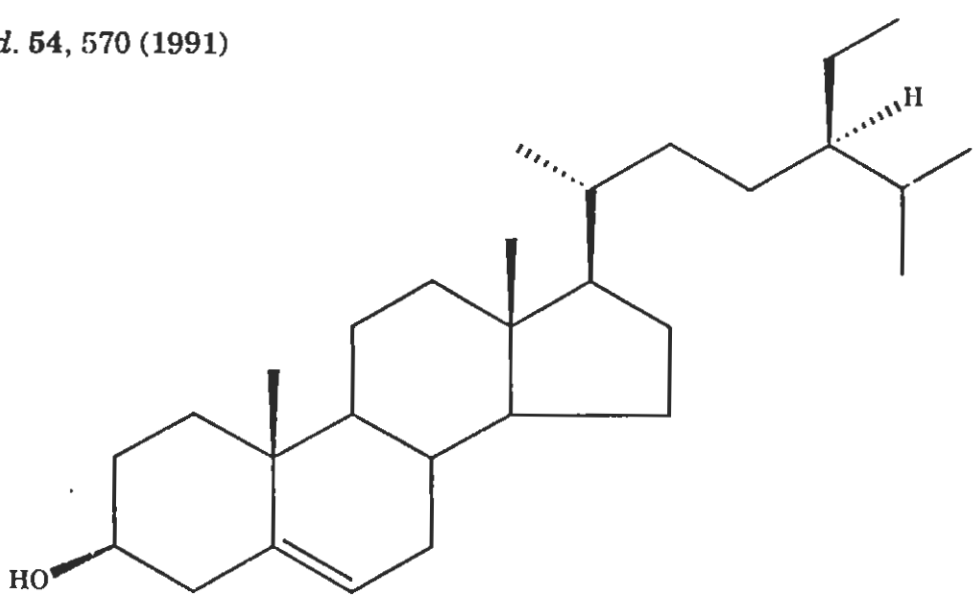

GENERAL INTRODUCTION

The art of healing has its origin in the antiquity of human civilization. The substances like henbens poppy, garlic and onion were used by the mankind even 6000 years ago for various medicinal purposes. The available literature references show that the first physician was evidently Chen Nung [1-2], emperor of China around 3000 B.C., who recorded a number of drugs and poisons through experiments on his own person, the results of which are presented in a compilation known as "Pen Tsau"-"The Great Herbal". He was the first one to note the diaphoretic and stimulatory effect of the drug "Ma-Huang" from which almost 5000 years later Nagai [3] isolated the active alkaloid ephedrine. In almost the same period Selkhet Enanach in Egypt [4] also pursued studies in the medicinal uses of herbs for the treatment of various human ailments. The ancient civilizations of Sumer, Babylon and Moenjodaro also made sizeable contributions in the medicinal field. Later, during the Hellenic and Greco-Roman period, a great reservoir of medical knowledge was provided by world famous physicians like Hippocrates [5], Theophrastus [6] and Galen [7]. However, the most significant pharmacological compilation of the Greeks was authoritative text of Discordies [7]. After him, Pliny the elder wrote "Natural History" in 37 volumes. Galen wrote some 30 books on pharmacology along with his "galenicals" preparations.

Following this period, the role of medicine was greatly extended in the Islamic age of science with passionate zeal and devotion. Among the famous name of this period are those of Rhazes credited with having written nearly 250 works some of which were on pharmaceutical subjects. His famous work is "Alhavi Kabeer". In this context it may also be noted that Avicenna is the world reknown author of "Canon" [8] which describes about 800 drugs and which surved as a standard text book of medicine in Europe till around the 17th century A.D., and continous as a base of the Greco-Arab system of medicine known as Tibb-e-Unanl. It is fairly

conceded by western scholars that the modern medical science had its origin in the Islamic culture of the middle ages, and eventually led to the efflorescence of medical technology in Europe in the wake of renaissance.

In the Indo-Pak sub-continent, records of the indigenous system of medicine go back to 700 B.C., and the systematization of Hindu medicine is attributed to Charaka [9] and Sasruta [10], of whom the latter has cited around 700 medicinal plants. In the last decade of the nineteenth century, Dymock, Hooper and Warden [11] published Pharmacographia Indica in three volumes which still serves as a classical work of reference on the medicinal plants of this region, providing comprehensive information on the botanical, chemical and pharmacological aspects of the then available knowledge in this field on the one hand, and forming a bridge between the modern and indigenous systems of medicine on the other. It may be stated in this context that the systems of medicine known as Unani-Tibb and Ayurveda have continued to provide medical relief in the Indo-Pak subcontinent. This may be considered as anachronism, but the practitioners of the Greco- Arab system of medicine ascribe to the drugs of their pharmacopoeia the advantage of extremely low toxicity, and the richness of experience in their therapeutic properties gathered since ancient times and systematised by no less a person than Avicenna. It was in recognition of these facts that systematic investigations in the constituents of indigenous medicinal plants were started in the 1920s at the School of Tropical Medicine in Calcutta, under the leadership of Colonel R.N. Chopra. However, these studies were mainly oriented towards the pharmacological aspects of plant materials. Following this initiative, studies in the chemical constituents of indigenous medicinal plants were carried out at various research centres and served the dual purpose of broadening the pharmacoplal spectrum and providing a basis of therapeutic studies directed towards the

synthesis of drugs modelled on the chemical structure of the natural products. In this context it is of special interest to note that organised systematic investigations in the chemistry of physiologically active principles of indigenous medicinal plants were first undertaken in 1930s at the Tibbi College, Delhi, established by the great physician and statesman, Hakim Ajmal Khan.

Away from this exclusive reliance on natural products in medicine, Paul Ehrlich [12] laid the foundation of chemotherapy in early 1890's. In the course of his researches in the field, the synthesis of salvarsan in 1907 by Ehrlich and Bertheim [13] fairly established the vital importance of this direction of work. He introduced [14,15] the concept of selective toxicity and provided evidence to the effect that the action of a drug depends on specific chemical combination with the parasite. His study [16] of selective dye staining for antiprotozoal activity and urinary antisepsis with azo dyes, along with selective staining as bacteriostatic and bacteriocidal measures, led to the study of certain dye groups as antimicrobials.

With the accelarated development of chemotherapy during the early decades of the current century and the phenomenal successes achieved with it, there has been an increasing shift from studies in natural products to synthetic drugs. Though this position has not materially changed, certain recovery of interest in medicinal plants was recorded as a result of the isolation of a whole series of new alkaloids from the roots of Rauwolfia serpentina which have gained world wide importance in the treatment of cardiovascular diseases and mental ailments. For instance, reserpine [17-23] has been extensively used in the treatment of hypertension and as an aid to psycotherapy. Ajmaline [24-28] on the other hand, has been used in many countries as a drug of choice against cardiac arrythmias of various origins. Subsequently the discovery of the life saving drugs of Vinca rosea,

vinblastine [29-34] and vincristine [31,35-36] for the treatment of certain forms of cancer, like Hodkin's diseases and leukaemias have further strengthened interest in researches relating to active constituents of medicinal plants. These investigations have served the dual purpose of bringing up new medicinally important substances and providing a basis of therapeutic studies directed towards the synthesis of drugs modelled on the chemical structure of the natural products.

The most frustrating feature in research efforts at the isolation of physiologically active principles of indigenous plant materials and the elucidation of their chemical structure and therapeutic status, has been the lack of institutionalised cooperation between botanists, chemists, pharmacologists, and clinicans, because it is only throug such cooperation that new therapeutic agents can be established. It is infact in consequence of gave back of it that drugs of plant origin have failed to acquire the full measure of their importance in the pharmaceutical industry. In this context, a recent authoritative survey of the American pharmaceutical industry can be referred in which it was brought out that 47.2% of the total number of prescriptions in United States during 1960 were based on natural products while 52.8% on synthetic drugs. Out of 47.2 % of the category of natural drugs, however, antibiotics derived from microorganisms made for as high a figure as 42.8% leaving a bare of 4.4% to physiologically active plant products and their derivatives.

While a great deal of good has certainly resulted from this trend in the alliviation and preventation of diseases, it may yet be stressed that the indifferent treatment accorded to the study of drugs of plant origin and insistence on synthetic drugs with the influx of proprietory medicines in all their baffling kaleidoscopic variations,

has not been wholly in the interest of human welfare.

There is yet another point that may be stressed here in justification of well planned systematic studies in field in a big way. Whatever has so far been done by way of researches in drug plants sums up to little more than scratching the surface of the problem, when one takes into account the fact that according to a recent survey, hardly 2% of the 191,000 species of flowering plants have been examined for alkaloids, let alone the other important chemical categories of active constituents. This may appear a baffling task, but certainly no more baffling than the task of those who have devoted themselves to the study of synthetics, because on an average around three to four thousand synthetic substances have to be prepared and pharmacologically screened before arriving at one with any therapeutic value, and even that lucky find has usually a short-lived career.

From the foregoing considerations it is quite evident that studies on medicinal plants continue to offer a vast and attractive field of research for chemists and presently surve as the mainstay for providing a big reservoir of physiologically active constituents for human welfare. The H.E.J. Research Institute of chemistry has been established in Karachi under the supervision of two world famous scientists namely Prof. Salim-uz- Zaman Siddiqui and Prof. Att-ur-Rehman with the provision of all possible modern sophisticated facilities for carrying out bioassay directed isolations and structural elucidations of natural products.

Taking into account the facts stated above the work under taken for the present doctoral desertation relates to the isolation and structural studies of new natural products from the two indigenous medicinal plants. The thesis is therefore divided into two parts entitled:

PART - A

PHYTOCHEMICAL STUDIES OF EUPHORBIA CLARKEANA

PART - B

PHYTOCHEMICAL STUDIES OF SERICOSTOMA PAUCIFLORUM

The introduction of "Part-A" provides a comprehensive review of all the compounds isolated so far from the Euphorbia species. It also gives a concise account of the biosynthesis of terpenes which appeared to be the principle constituents of both the species.

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PART - A

INTRODUCTION

It has been known for many centuries that the fragrant odours possessed by many plants are associated with volatile liquids which are known as the essential or volatile oils. They are also employed as solvents in paints, as preservatives and artificial flavorings in foodstuffs, and to a small extent in medicine. Interest in the chemical constitution of the essential oils became widespread in the nineteenth century. Many of those were found to contain one or more of a series of isomeric unsaturated hydrocarbons of formula $C_{10}H_{16}$, which came to be known as "terpenes", from their association with oil of terpentine. A number of oxygen containing derivatives of the terpenes were also isolated from plants. Early structural investigations on the $C_{10}H_{16}$ terpenes revealed that those were dimers of the compound "isoprene". Later, a vast number of terpenes were isolated from a large variety of plants whose molecular formula was integral multiple of $C_{10}H_{16}$. Thus we can define terpenoids as the natural products which possess a carbon framework containing two or more isoprenic units of five carbon atoms. This immediately led to a rational classification of the terpenes depending upon the number of such isoprene units. They are monoterpenes ($C_{10} = 2$ X C_5), sesquiterpenes ($C_{15} = 3$ X C_5), diterpenes ($C_{20} = 4$ X C_5), sesterpenes ($C_{25} = 5$ X C_5), triterpenes ($C_{30} = 6$ X C_5), and tetraterpenes ($C_{40} = 8$ X C_5).

BIOSYNTHESES OF TERPENOIDS

The biosynthesis of terpenoids may be studied under three subheadings.

1. Biosynthesis of isopentene unit.

2. Condensation of isopentene unit to acyclic terpenoids.

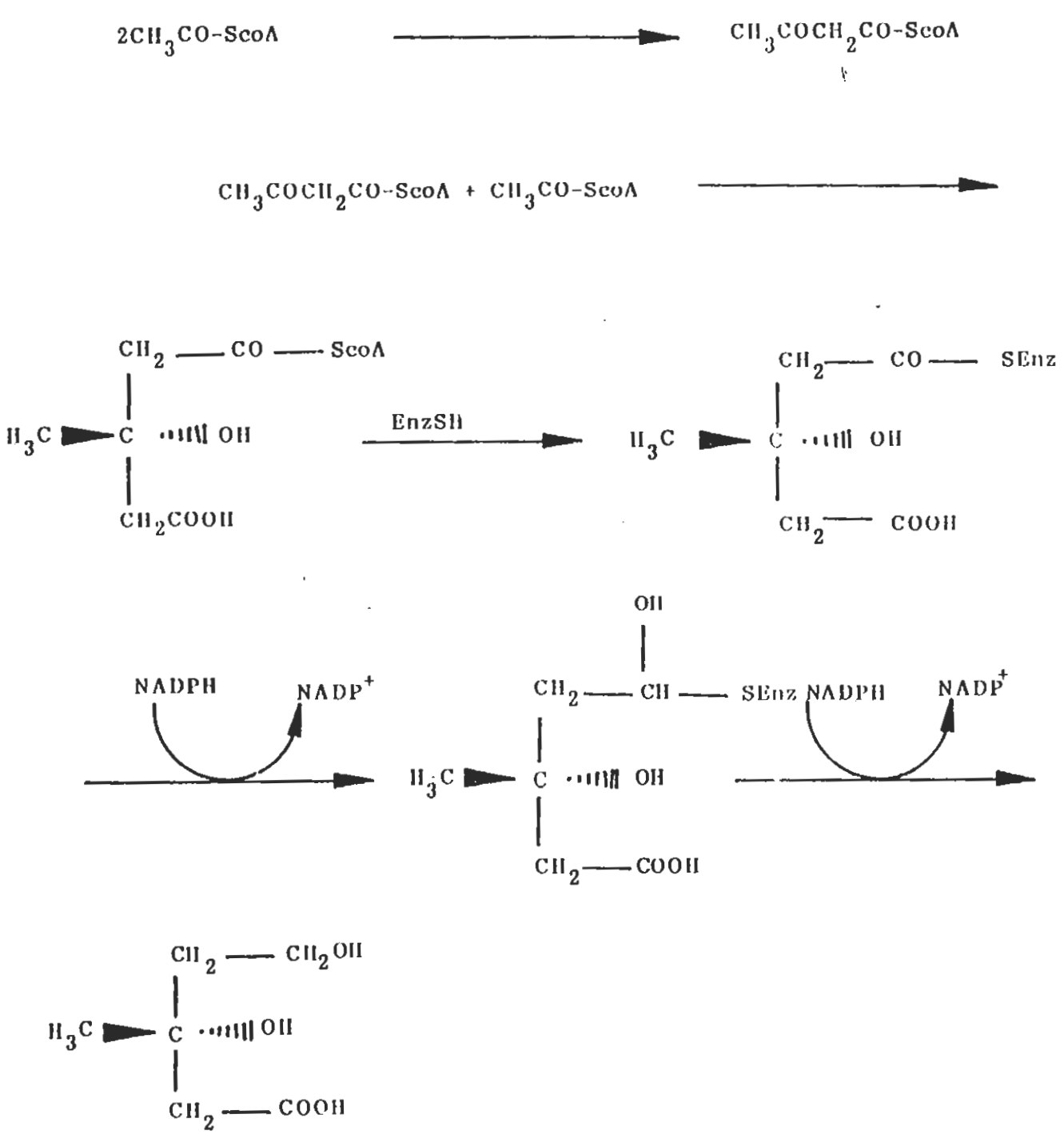
3. Cyclisation of acyclic terpenoids.

1. Biosynthesis of Isopentene Unit.

The acetate ion in the form of acetyl-coenzyme A derived mainly from carbohydrate and fat metabolism is generally considered to be converted into mevalonic acid (**1**) via acetoacetyl co-enzyme A and S-3-hydroxy-3-methyl-glutaryl-CoA. The latter is reduced irreversibly to R-mevalonic acid (**1**) by the hydrogen which transfers from reduced nicotine amide adenine dinucleotide phosphate (NADPH) (Scheme-1). Only R-form mevalonic acid is utilized by organisms for producing terpenes, while S-form is metabolically inert.

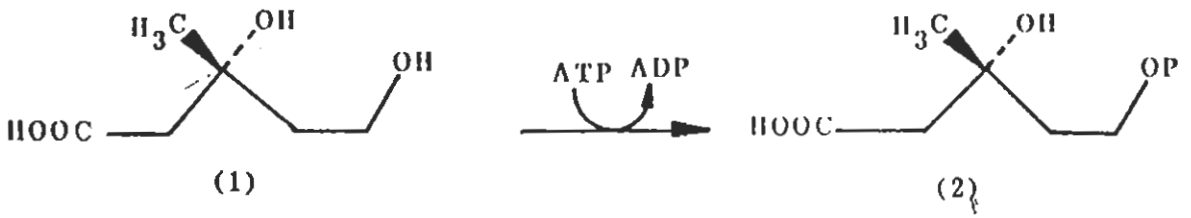
The (R)-mevalonic acid is converted into isopentene by the route shown in Scheme-2 which requires the loss of one carbon atom. The isotope experiments have shown that it is the carboxylic acid which is lost [1-8]. Phosphorylation of mevalonic acid first produces mevalonic acid-5-phosphate (**2**) which is followed by further phosphorylation to give mevalonic acid-5-pyrophosphate (**3**). The whole process requires 2-molecules of adenosine triphosphate (ATP). Subsequent loss of a water molecule and carbon dioxide yields 3-methylbut-3-enyl pyrophosphate or D^3-isopentenyl pyrophosphate (IPP) (**4**).

The IPP can undergo an isomerisation into 3,3-dimethylallyl pyrophosphate (DMAPP) (5) [8]. The consequence of this isomerisation is to transform a relatively unreactive substance into a reactive molecule capable of attacking nucleophilic species. This isomerisation is stereospecific. The enzyme involved selectively removes $^*H$ and the newly formed methyl group takes trans orientation with respect to the ester group (Scheme-2).

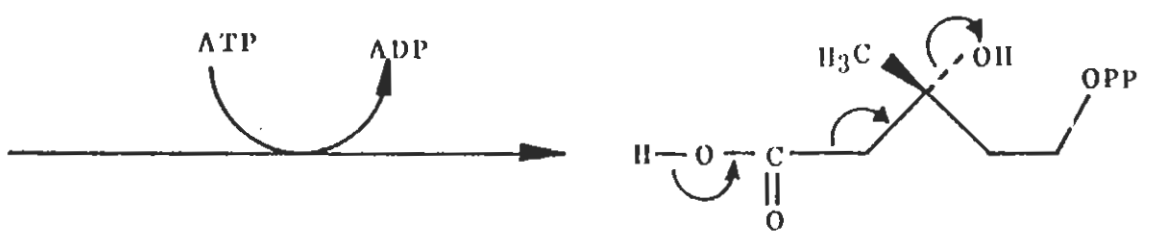


(R)-Mevalonic acid (1)

Scheme-1

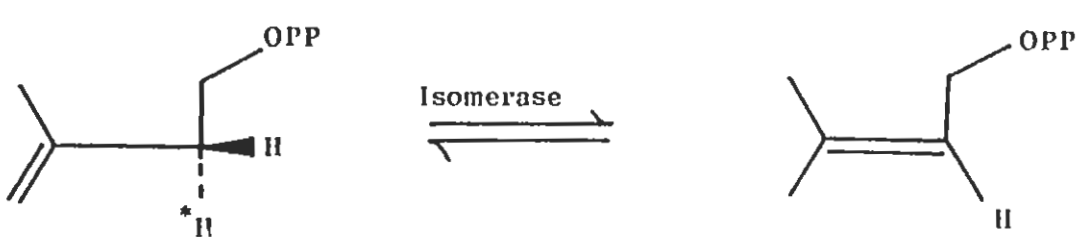


(1) (2)



(3)

IPP (4)



IPP (4) DMAPP (5)

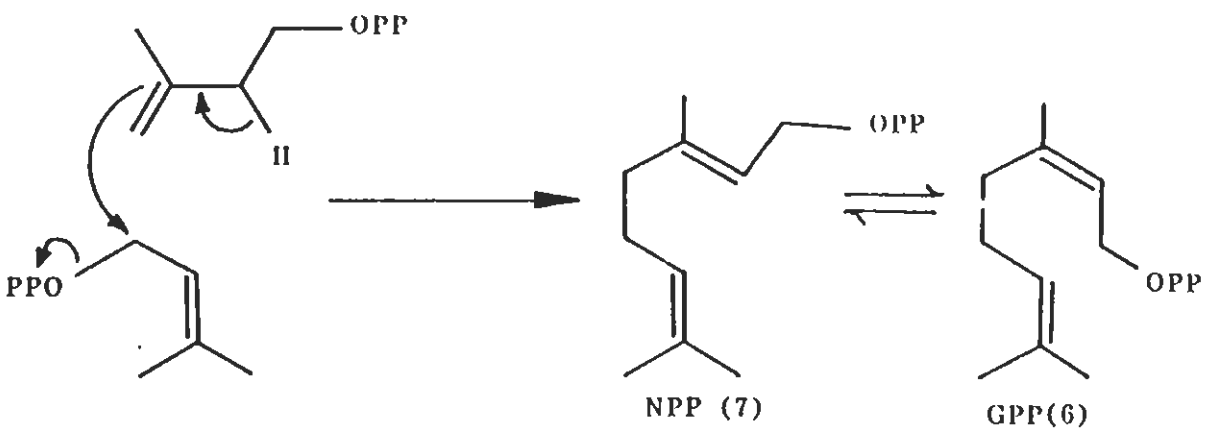
Scheme-2

2. Condensation of Isopentene Unit to form Acyclic Terpenoids.

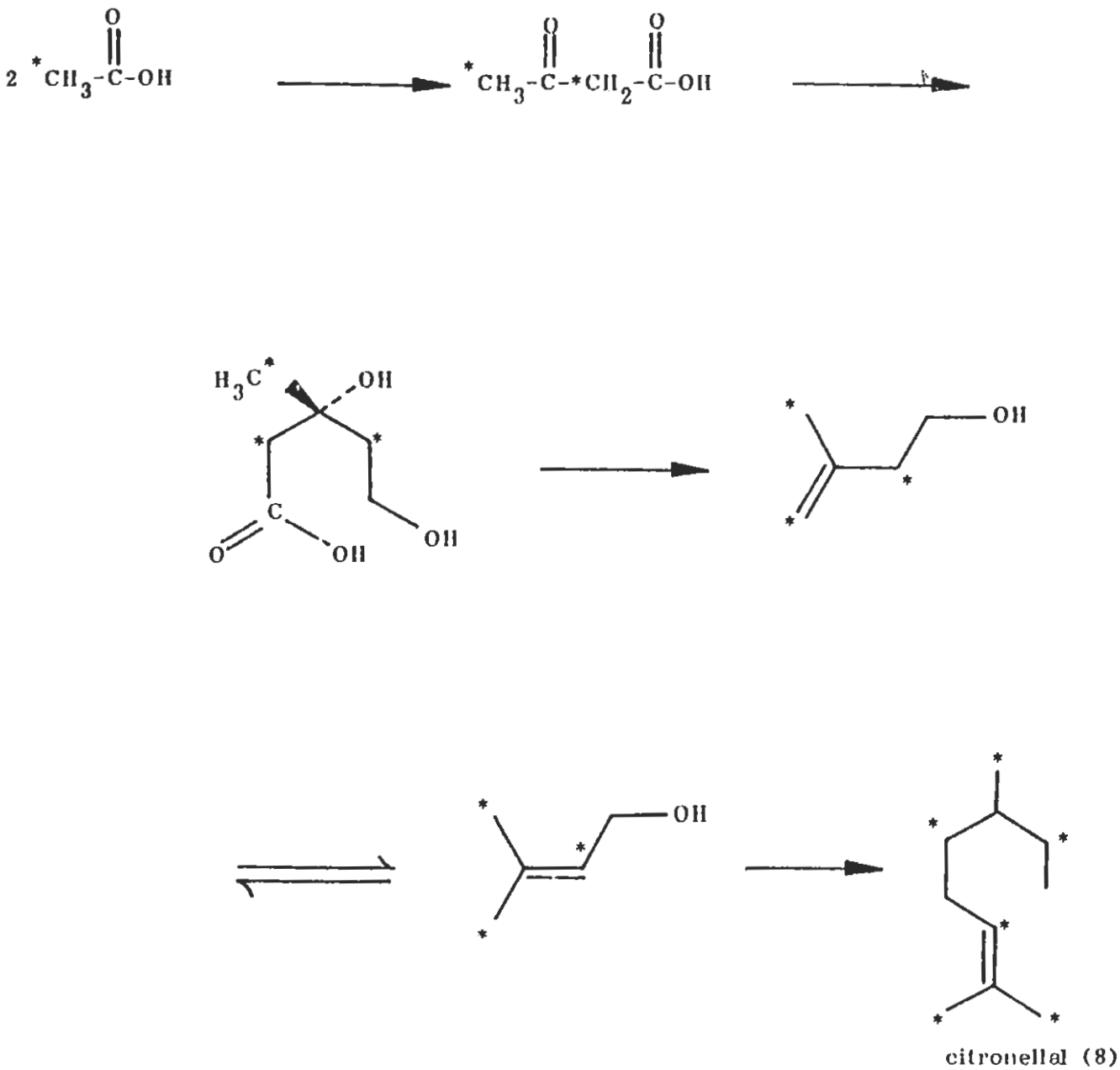
The DMAPP molecule (5) can condense in a head-to-tail manner with IPP (4) by a formal combined S_N2 - E2 process to give geranyl pyrophosphate (GPP) (**6**) and neryl pyrophosphate (NPP) (**7**) (Scheme-3). This route is supported by biosynthetic experiments with labelled acetate leading to citronellal (**8**) (Scheme-4). IPP can further condense with GPP to give farnesyl pyrophosphate (FPP) (**9**) in all trans form (Scheme-5) [9-13]. It is considered to be the direct precursor of sesquiterpenoids, which in turn can dimerize in a tail-to-tail fashion to give squalene (**10**) (Scheme-5) [14-15], the immediate precursor of triterpenoids. FPP can add another C_5 unit to form gerenyl geranyl pyrophosphate (GGPP) (**11**) (Scheme-5) [16] which is cyclised to give diterpenes.

3a. Cyclization of Acyclic Terpenoids to Diterpenes.

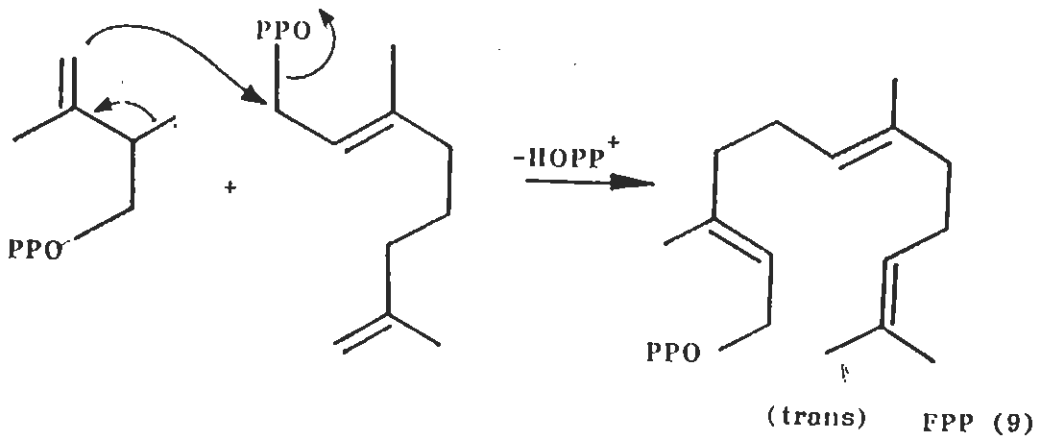
The biogenesis of the cyclic terpenoids reveals two fundamentally different types of cyclization reactions. In the first, the pyrophosphate acts as a leaving group to initiate the primary cyclization by generating a carbocation which then alkylates one of prenyl double bonds. In the other, cyclization is initiated by protonation of a double bond or its corresponding epoxide. In both instances these primary cyclizations are often followed by a series of secondary cyclization and rearrangement reactions. The diterpenoid reveal examples of both pathways.



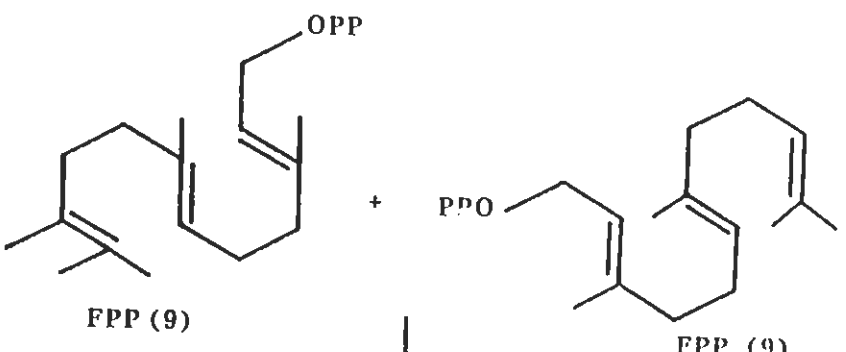
Scheme-3



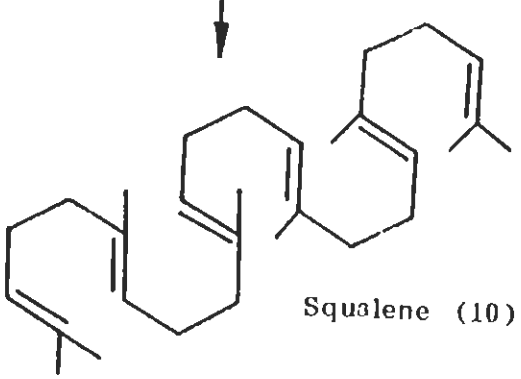
Scheme-4



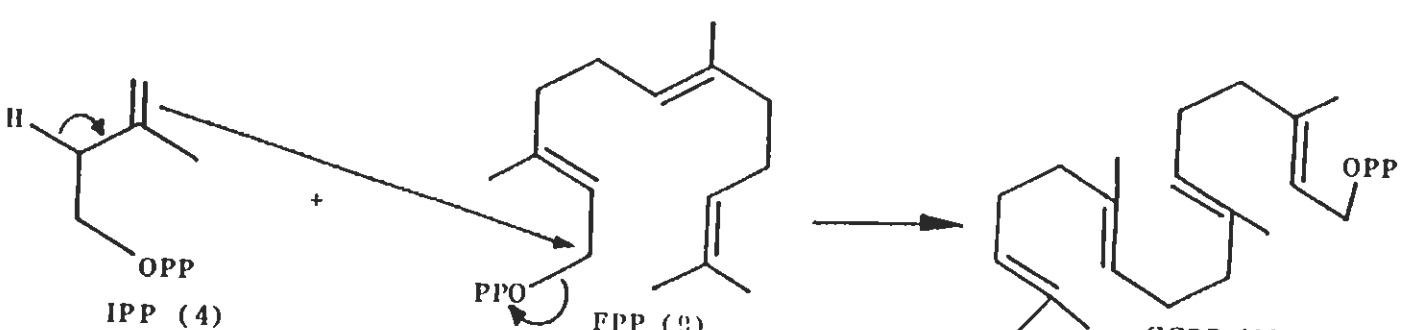
(trans) FPP (9)



FPP (9) FPP (9)



Squalene (10)



IPP (4) FPP (9) GGPP (11)

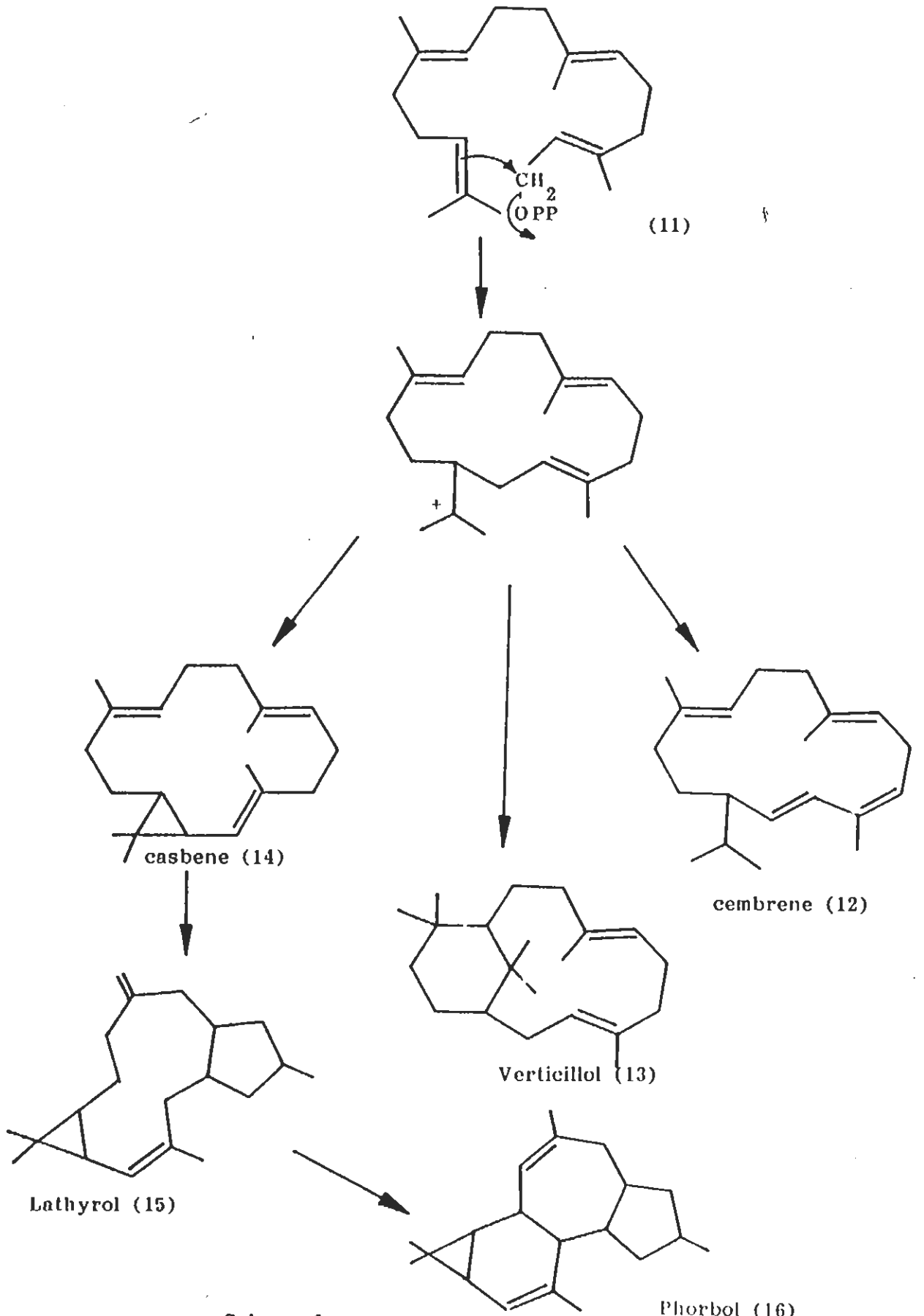
Scheme-5

In case of monocyclic diterpenes, cyclization is induced by the heterolytic cleavage of the CH_2-OPP portion of (**11**) generating a carbonium ion which then attacks the starter isopropylidene unit leading to cyclization. It has been suggested that cembrene (**12**) arises from GGPP (**11**) in this manner. Further cyclization and rearrangements of the macrocyclic ring may lead to carbon skeleton of verticillol (**13**), casbene (**14**), lathyrol (**15**) and phorbol (**16**) (Scheme-6) [16-18].

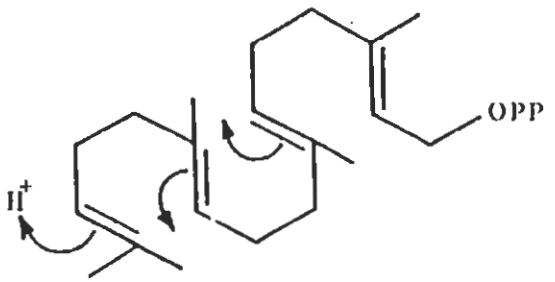
The biosynthesis of bicyclic diterpenes such as selereol (**17**), manool (**18**) and isomeric labdanes (**21,22**) are shown in Scheme-7. These are directly derived from the cyclization of GGPP (**11**). The junction between the ring A and B of all labdane diterpenes and in compounds with larger number of rings is invariably trans. The nature of this ring junction is a function of the relative orientation of the cyclizing double bonds in open chain precursor on an enzyme surface [7].

The conformation of the polyenic chain during the cyclization sequence is chair-chair (ring A-B), such a conformation can exist in two enantiomeric forms (**19**) and (**20**), from which the two enantiomeric labdane skeletons (**21**) and (**22**) derive. Since many labdane diterpenes are subsequently transformed in plants into tri-, tetra-, and penta-cyclic diterpenes, such compounds are also divided into the two enantiomeric series.

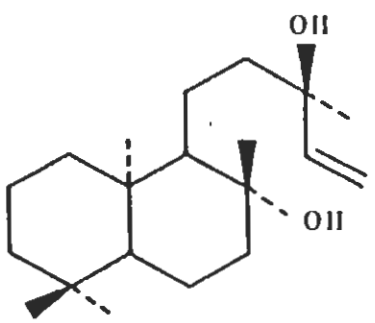
The tricyclic pimarane skeleton (**24**) can be derived by further cyclization of monool (**18**) through interaction between the incipient C-13 carbocation and the 8, 14-double


CH2
OPP
(11)
casbene (14)
cembrene (12)
Verticillol (13)
Lathyrol (15)
Phorbol (16)

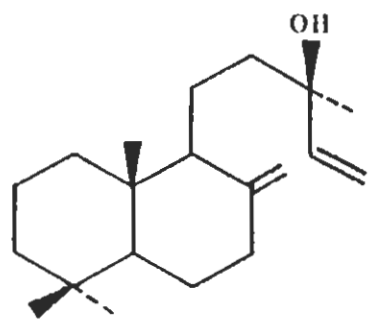
Scheme-6



GGPP (11)

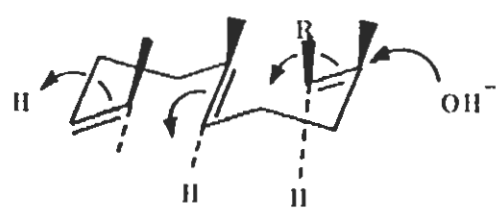


Sclereol (17)



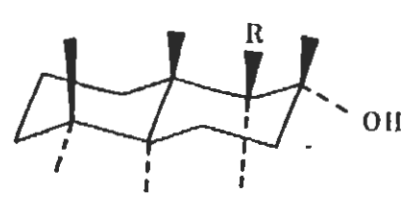
Manoöl (18)

(19)



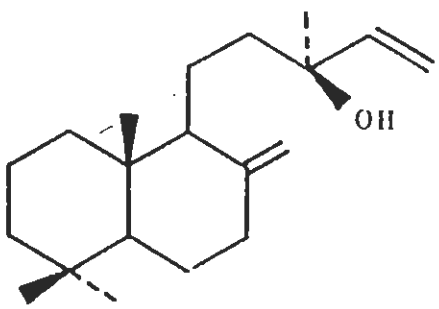
(20)

10Meα-Labdane (21)

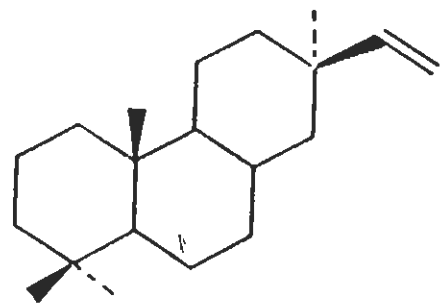


10 Meß-Labdane (22)

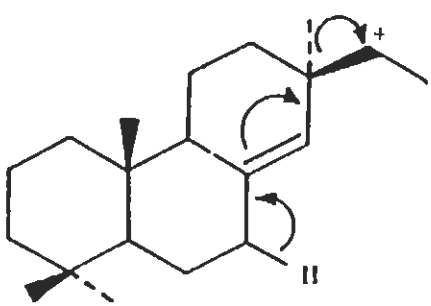
Scheme-7



Manoöl (18)

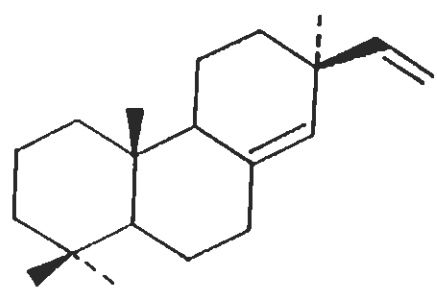


(23)

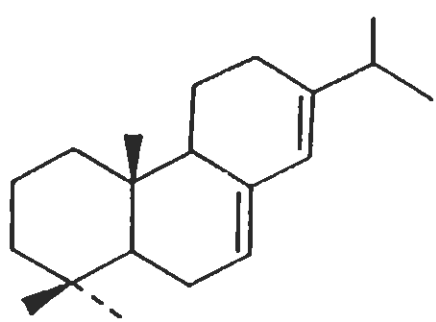


(25)

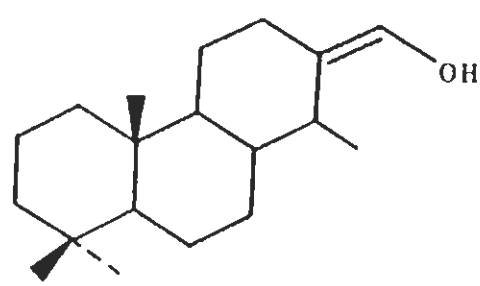
H^+



Pimaradiene (24)



Abietadiene (26)



Cassaine (27)

Scheme-8

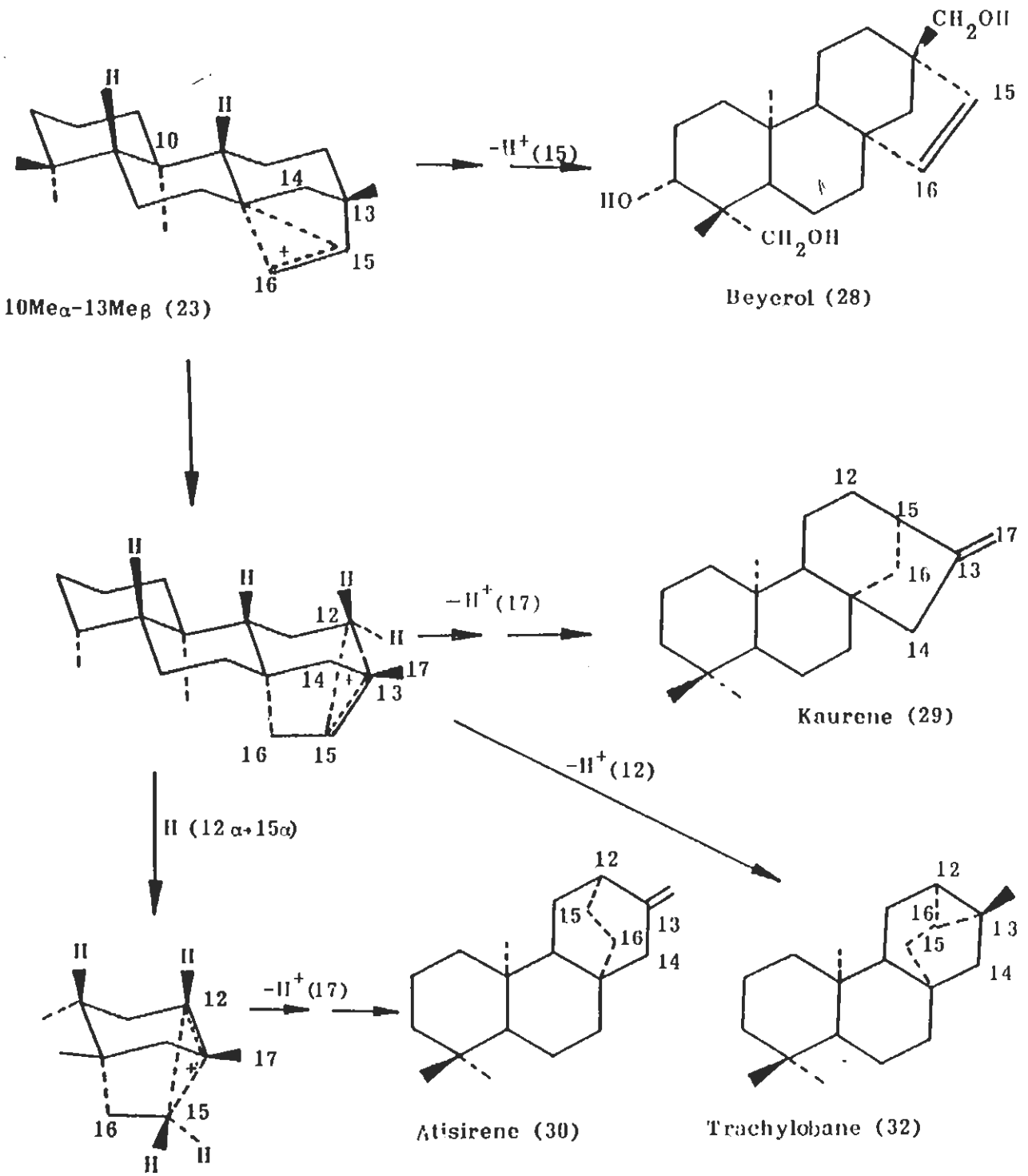
bond via intermediate (**23**). Rearrangement of the derived carbonium ion (**25**) gives the abietane (**26**) and cassaine (**27**) skeletons (Scheme-8) [19].

The tetra- and penta-cyclic diterpenes are closely related series of pimarane. These arise through non-classical pimarane cation (**23**) which can then collapse in a variety of ways leading to the beyerene (**28**), kaurene (**29**), atisirene (**30**), phyllocladane (**31**), and pentacyclic trachylobane (**32**) skeletons (Scheme-9) [20,7].

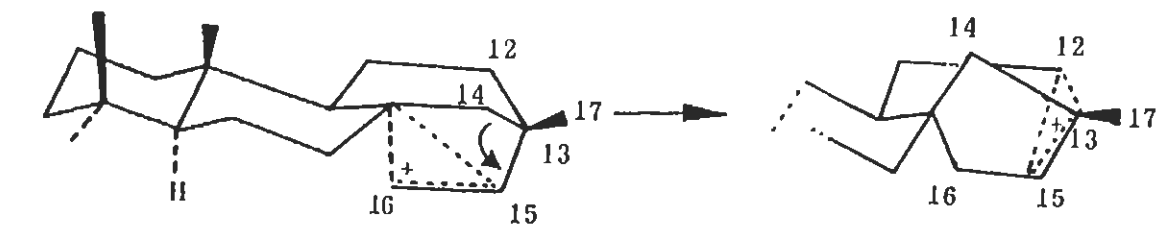
The diterpenes gibbane (**33**), grayanotoxane (**34**), and enmeane (**35**) belong to the tetracyclic series and possibly derive form the kaurane skeleton. During the rearrangement processes the bonds at positions 5 (**10**), 6(**7**) and **7**(**8**) are respectively broken (Scheme- 10) [6,21,22].

3b. Cyclization of Acyclic Terpenpoids to Triterpenoids.

The hexa-ene all-trans squalene (**10**) is the immediate biological precursor of all triterpenes. Its stereochemistry is established by an X-ray investigation of its urea adduct and it has also been synthesised recently by a number of methods [23-25]. The triterpenes which directly derive form the cyclization of squalene or of its 2,3-epoxide are called fundamental triterpenes. The latter can be divided into sub groups according to their cyclization path.

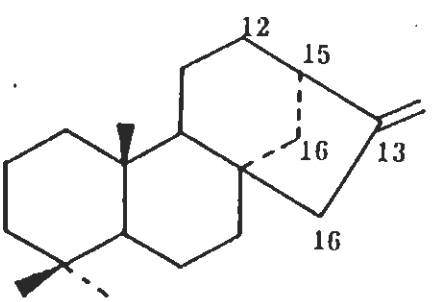

10Meα-13Meβ (23)
-H+(15)
Beyerol (28)
-H+(17)
Kaurene (29)
-H+(12)
H (12α→15α)
-H+(17)
Atisirene (30)
Trachylobane (32)

Scheme-9



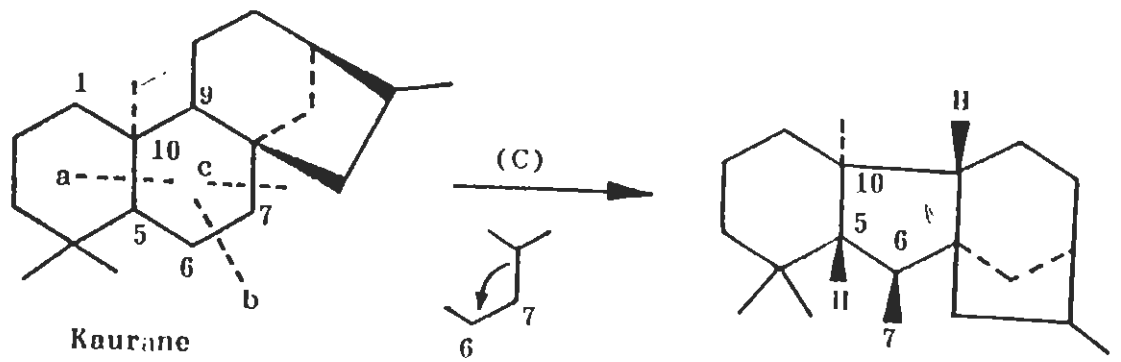
10 Meβ- 13 Mcβ (23)

$-H^{+}$ (17)



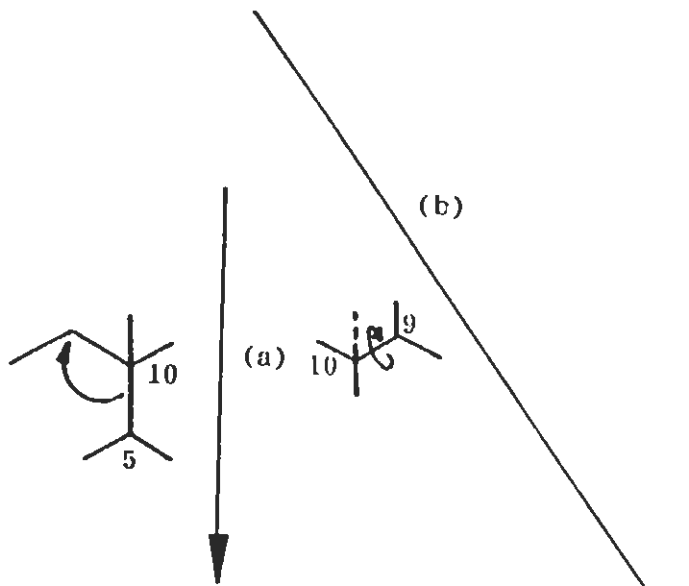
Phyllocladene (31)

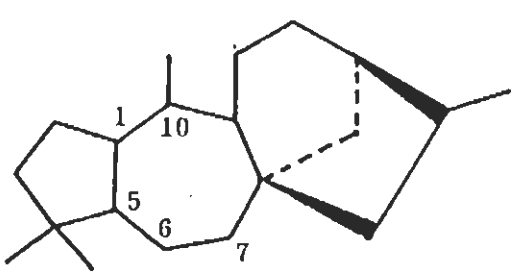
Scheme-9



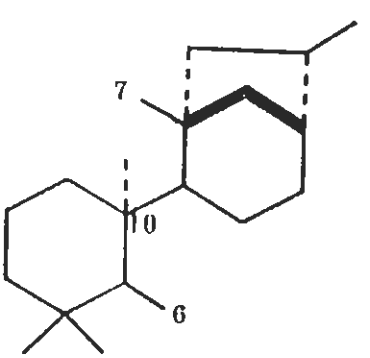
Kaurane

Gibberellane (33)





Grayanotoxane (34)



Enmeane(35)

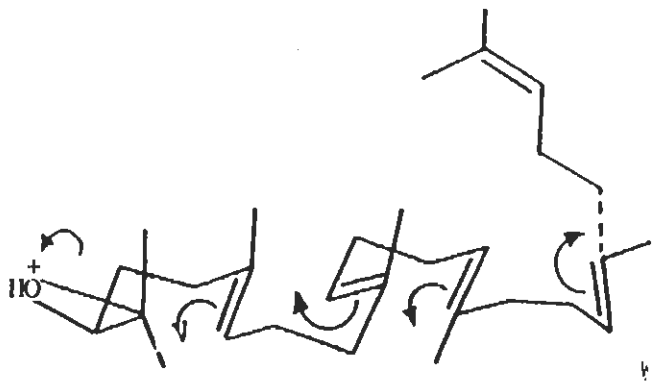
Scheme-10

OXIDATIVE CYCLIZATION ARISING FROM ONLY ONE TERMINAL PART OF SQUALENE.

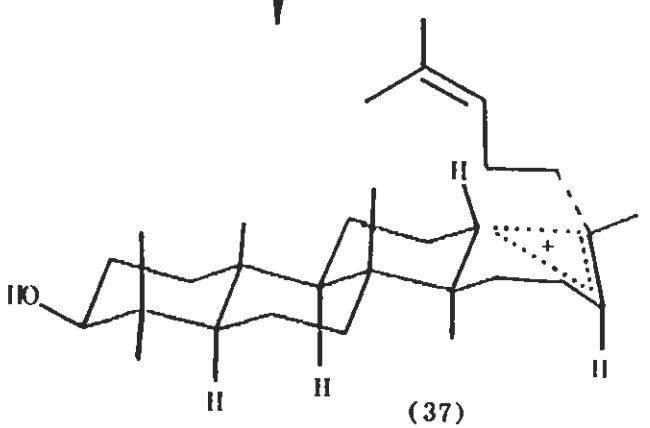
Different types of tetra- and penta-cyclic triterpenes are derived from this type of cyclization via 2,3-epoxy squalene (**36**) and are classified according to the conformation of squalene epoxide. Thus the chair-chair-chair-boat form leads via a smooth cyclization to tetracyclics including dammeranediol (**38**) [26,27], euphol (**39**) and tirucallol (**40**) [28,29] (Scheme-11,12) and pentacyclics such as lupeol (**43**), taraxasterol (**44**) [30], α-amyrin (**45**) [31], taraxerol (**46**) [32], β-amyrin (**47**) [33,34], friedeline (**48**), and δ-amyrin (**49**) [34] (Scheme-13,14). On the other hand, a chair-boat-chair-boat conformation (50) leads to lanosterol (**52**) and cycloartenol (**53**) (Scheme-15,16). Hopanone (**55**), arborinol (**57**) and moretenol (**59**) are formed by the concerted cyclization mechanism involving the whole 2,3-epoxy squalene molecule. The chair-chair-chair-chair conformation (**54**) gives hopanone (**55**), chair-boat-chair-chair-boat (**56**) gives arborinol (**57**) whereas chair-chair-chair-chair-boat conformation (**58**) leads to moretenol (**59**) (Scheme-17).

NON-OXIDATIVE CYCLIZATION ARISING FROM ONLY ONE TERMINAL PART OF SQUALENE.

Such cyclization route is less common then the oxidative path. Only few triterpenes such as tetrahymanol (**61**) [35] and diploptene (**62**) [36] arise by this type of cyclization (Scheme-18).

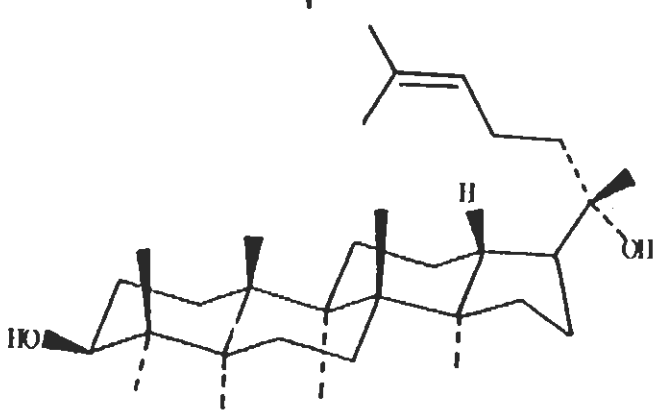


(36)



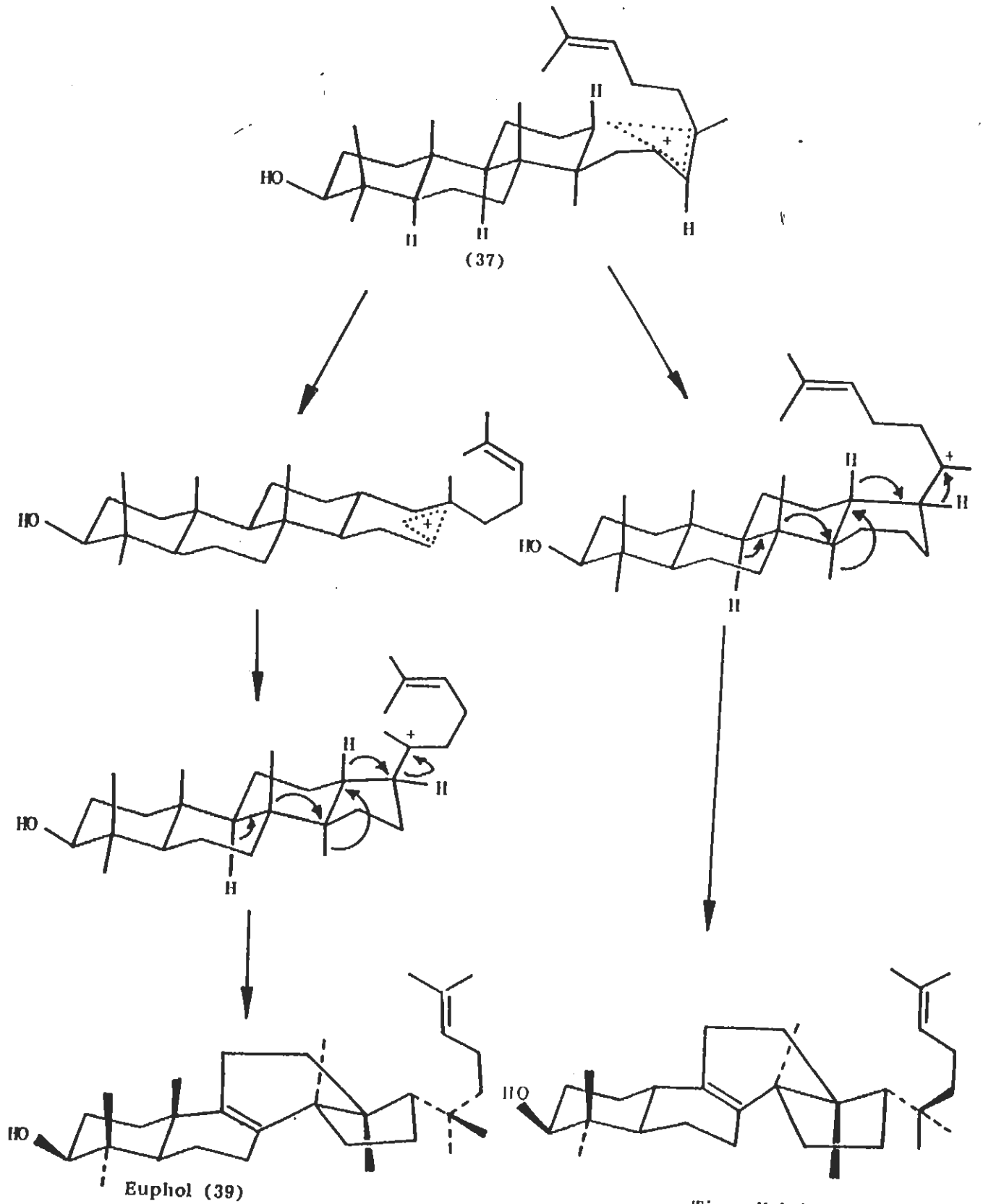
(37)

$\overline{O}H$

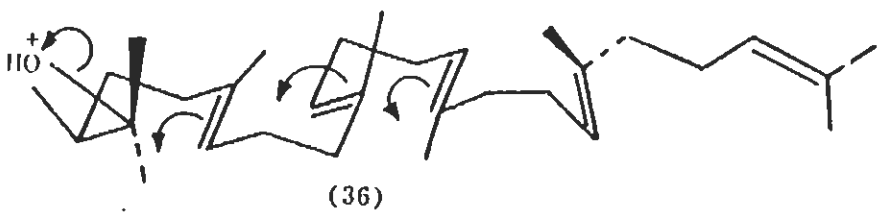


Dammerenediol (38)

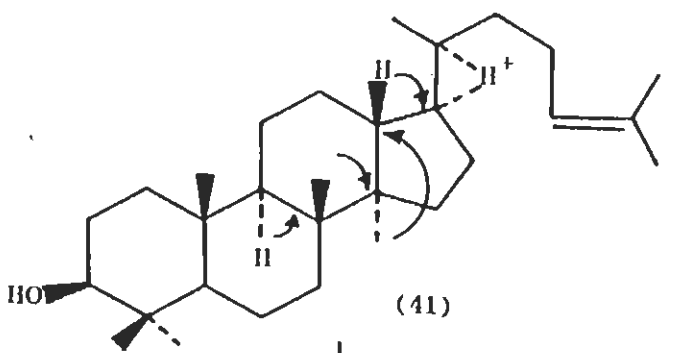
Scheme-11



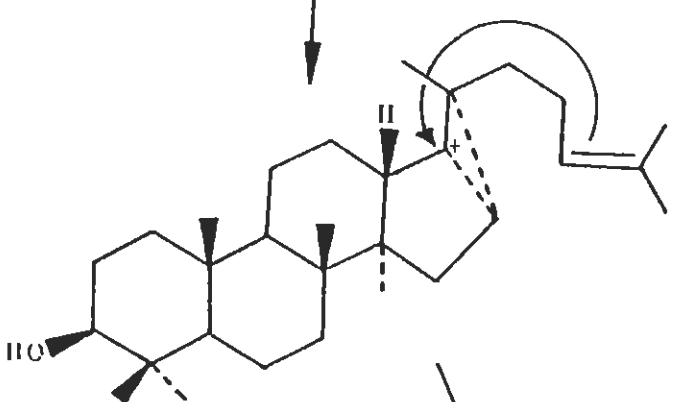
Scheme 12

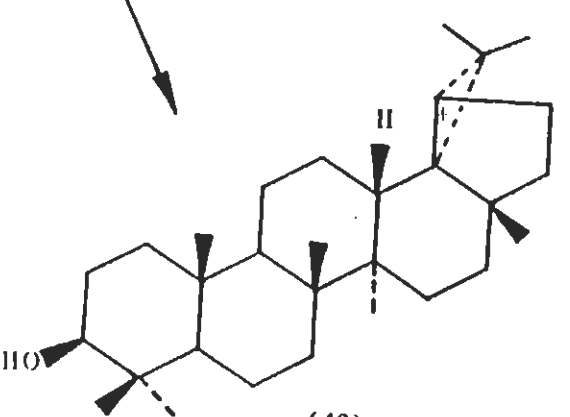


(36)

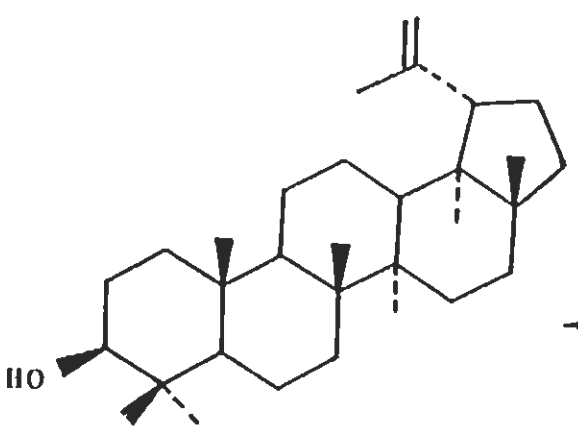


(41)





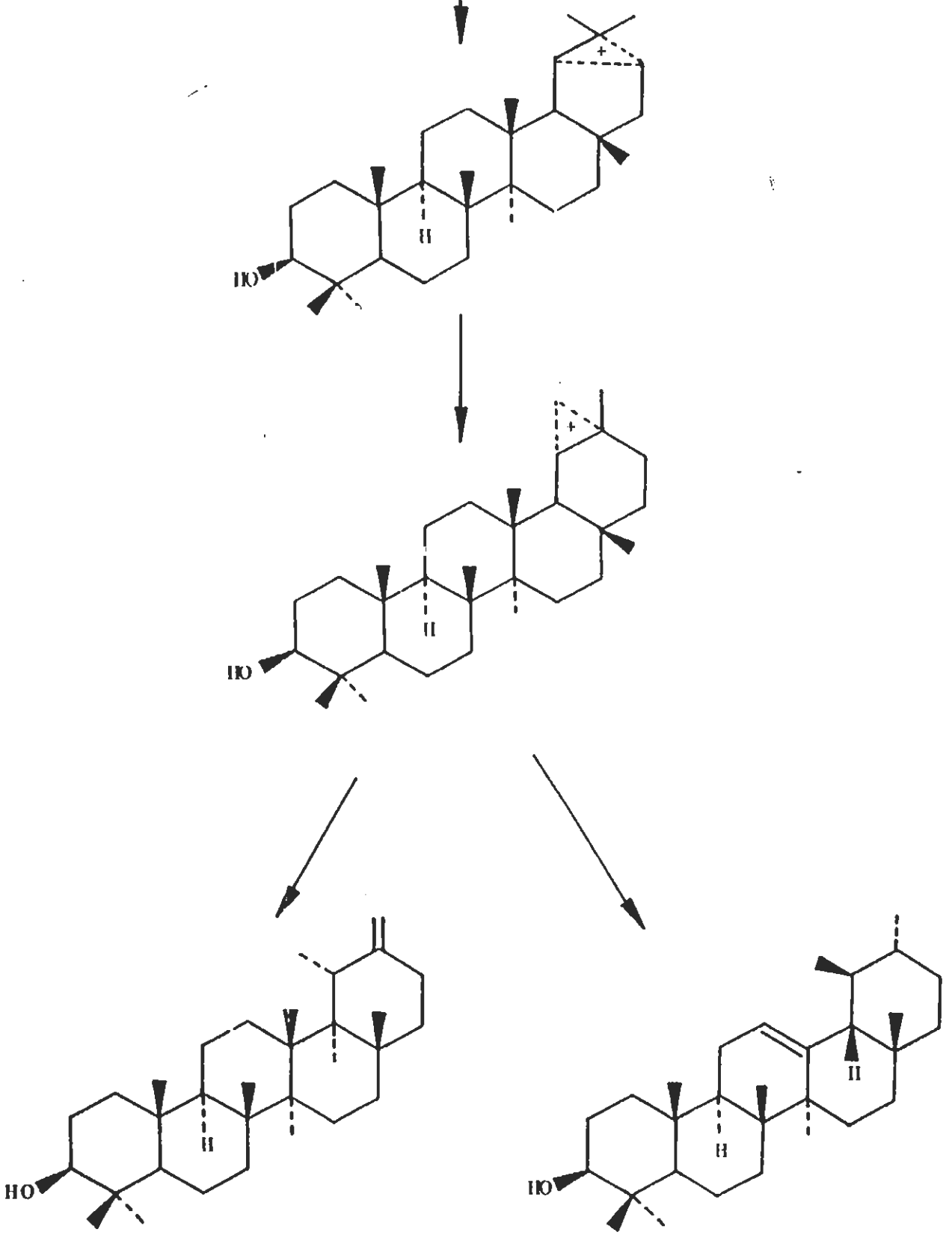
(42)



Lupeol (43)

Scheme-13

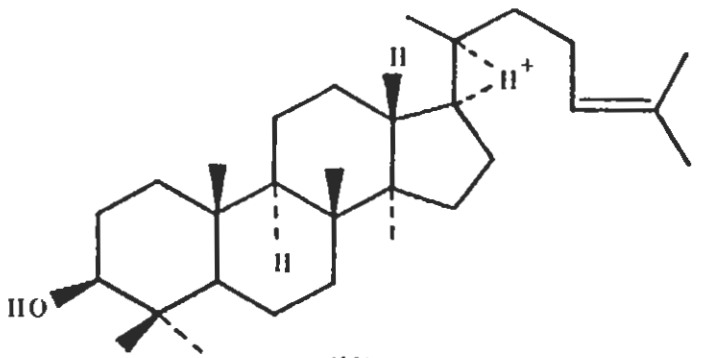
41



Taraxasterol (44)

α-Amyrin (45)

Scheme-13



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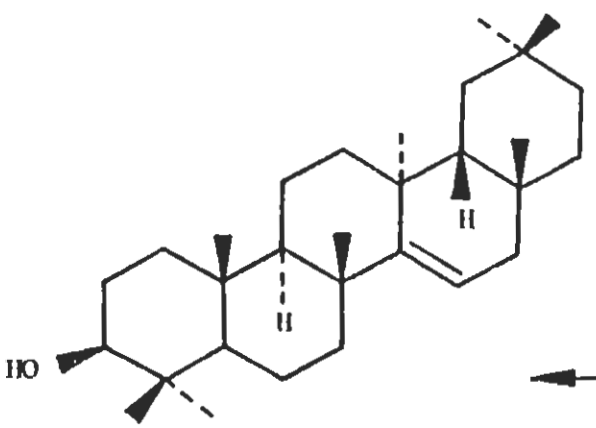
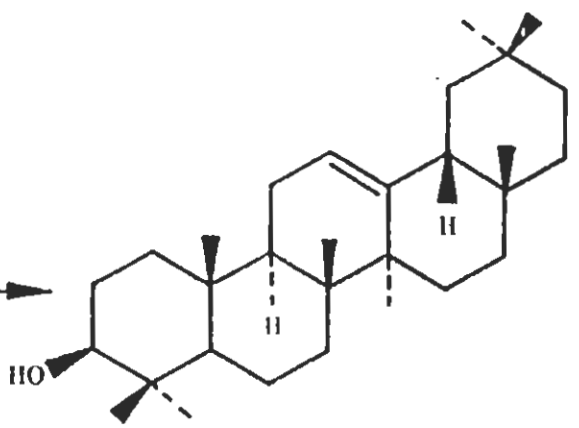
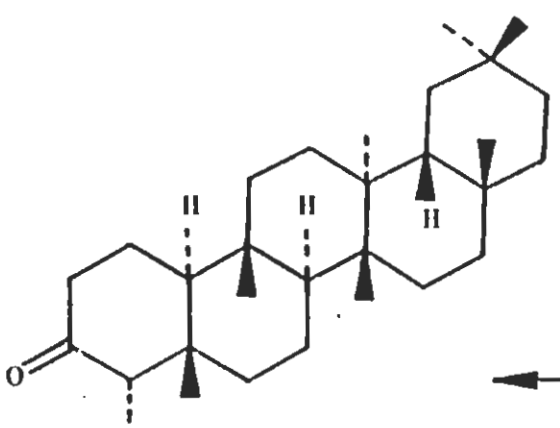


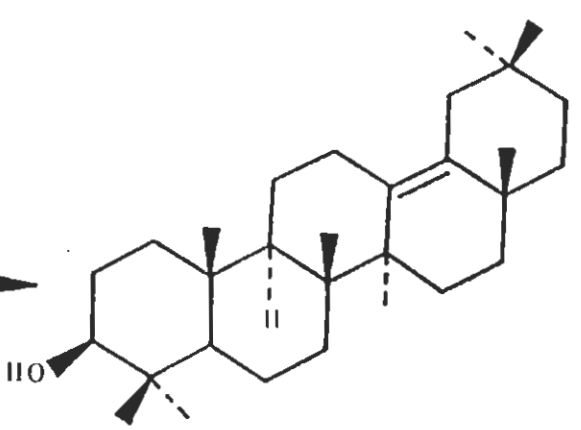
Taraxerol (46)



β-Amyrin (47)

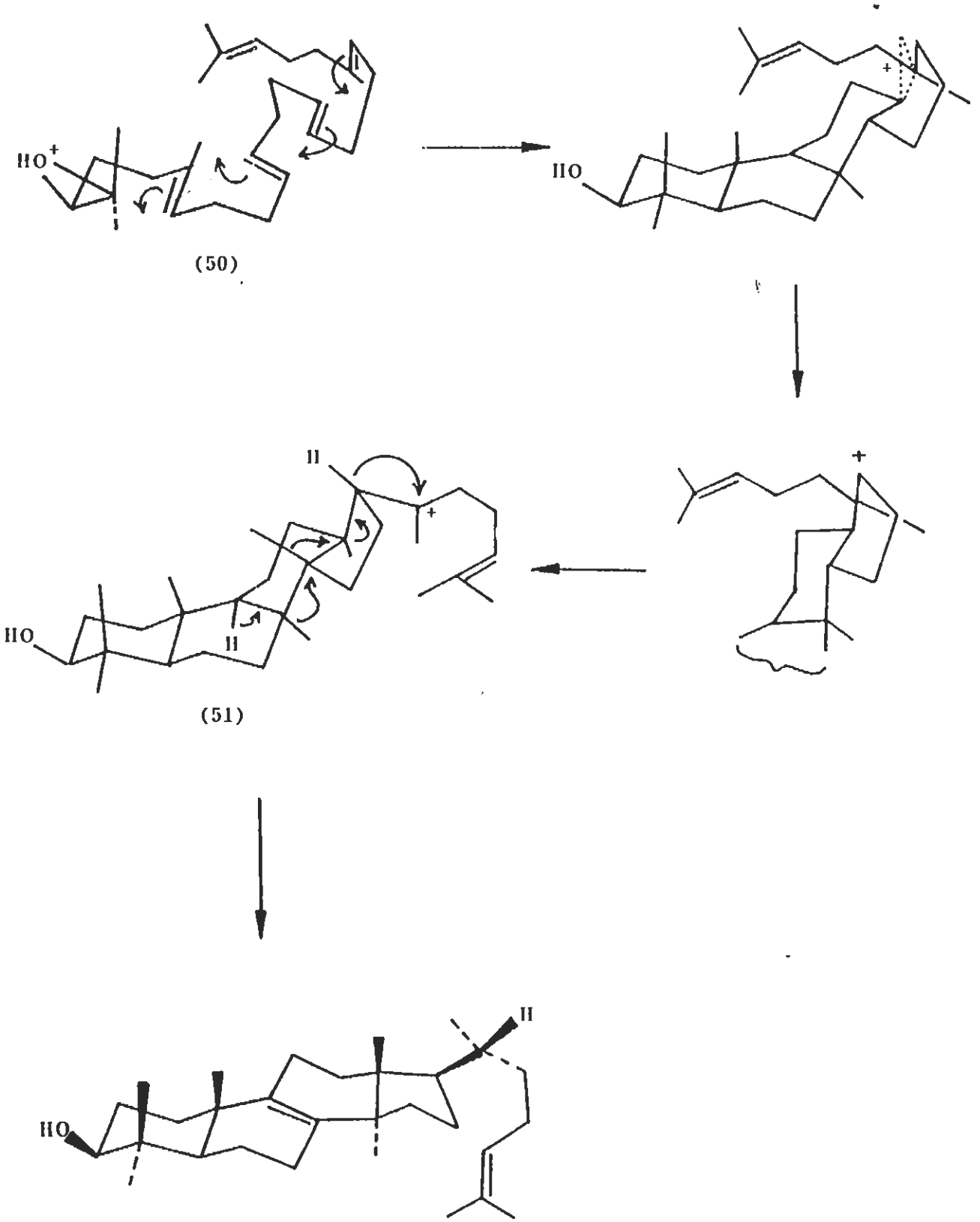


Friedeline (48)



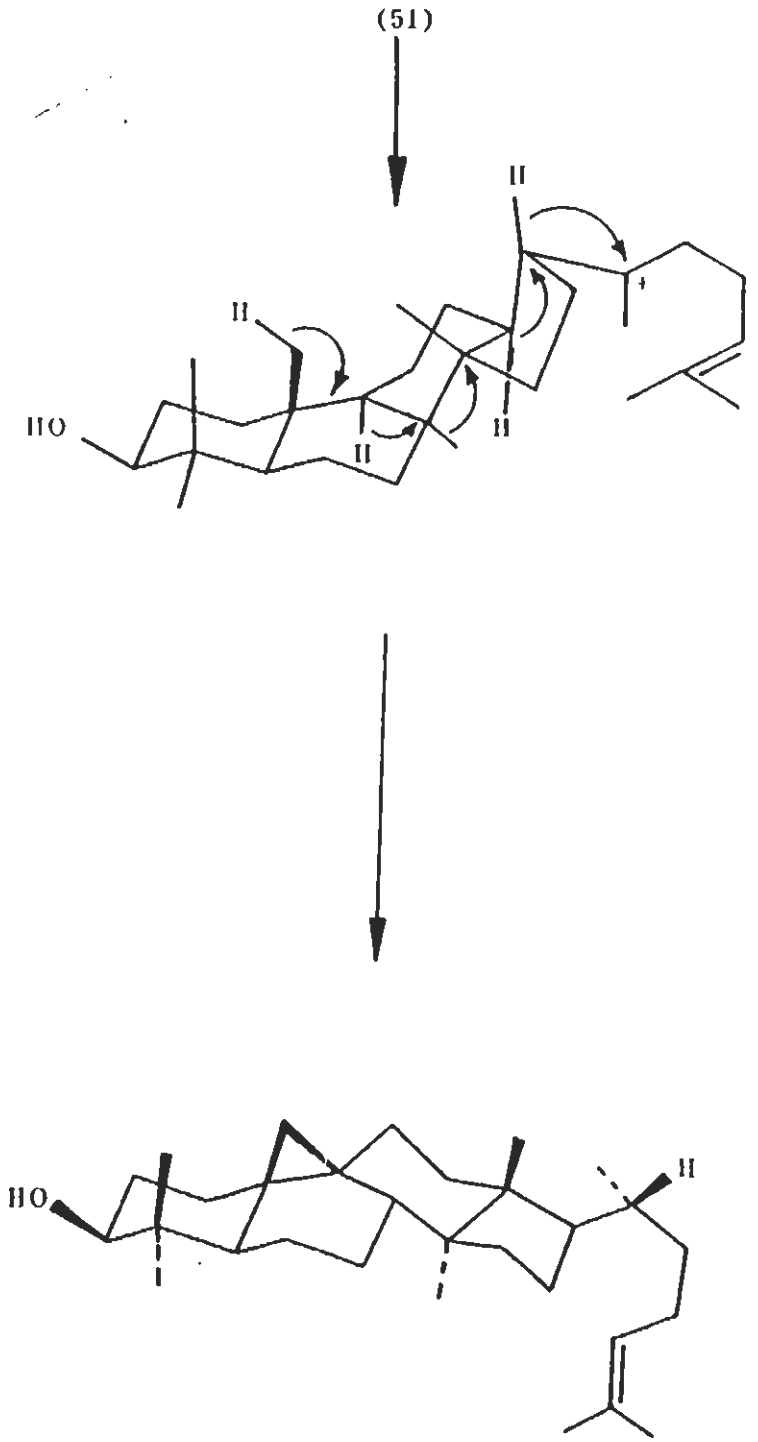
δ-Amyrin (49)

Scheme-14



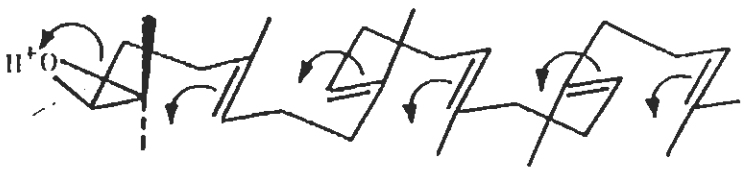
Lanosterol (52)

Scheme-15

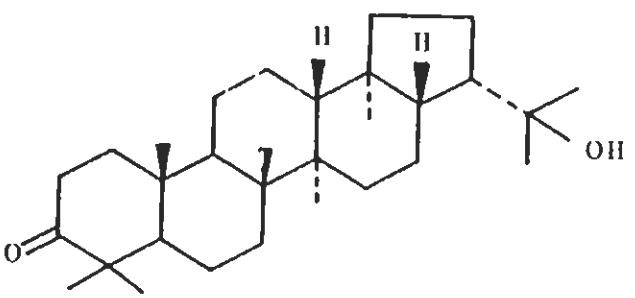


Cycloartenol (53)

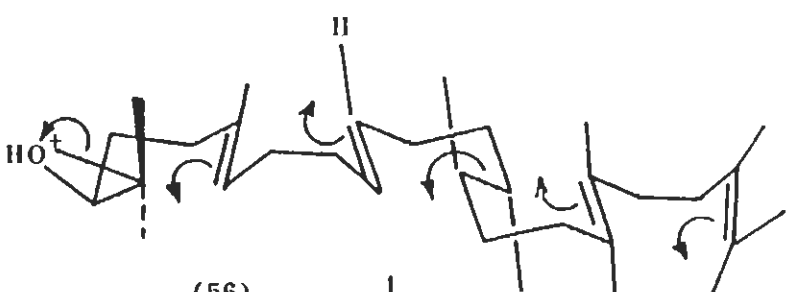
Scheme-16



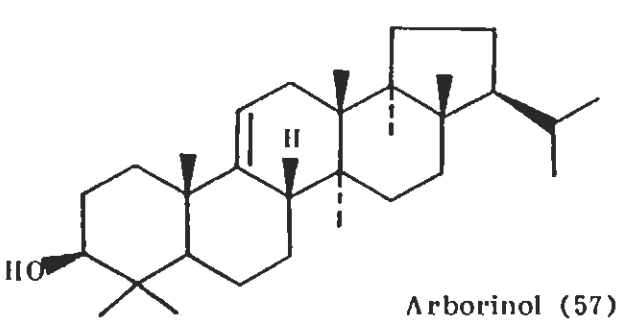
(54)



Hopanone (55)

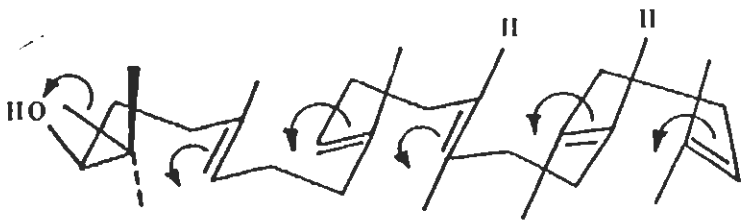


(56)



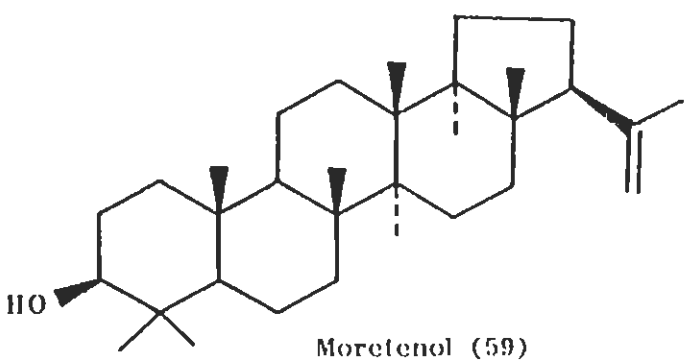
Arborinol (57)

Scheme-17 (cont'd.)



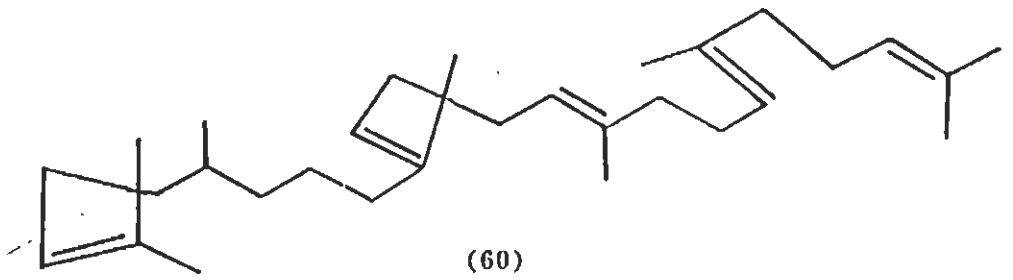
(58)

$-H^+$

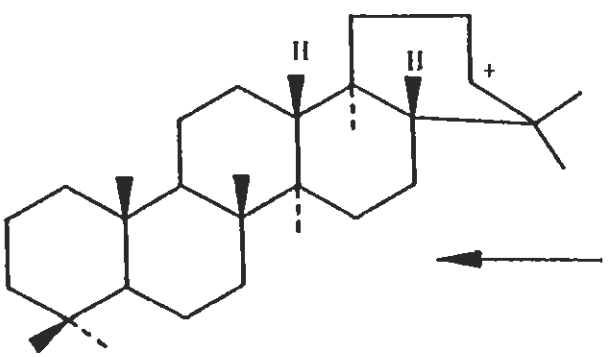


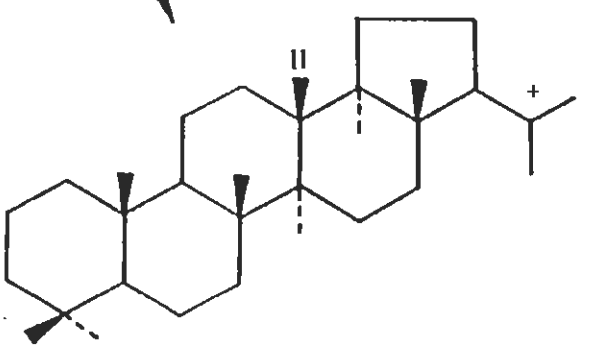
Moretenol (59)

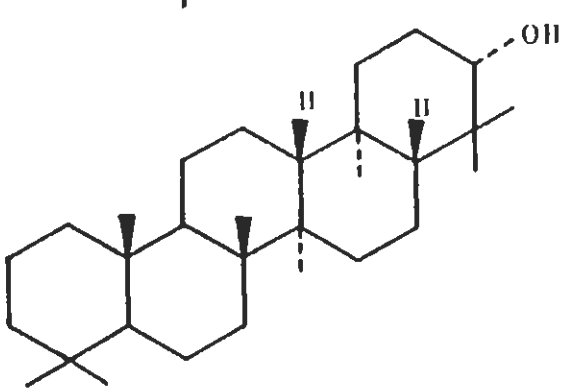
Scheme-17

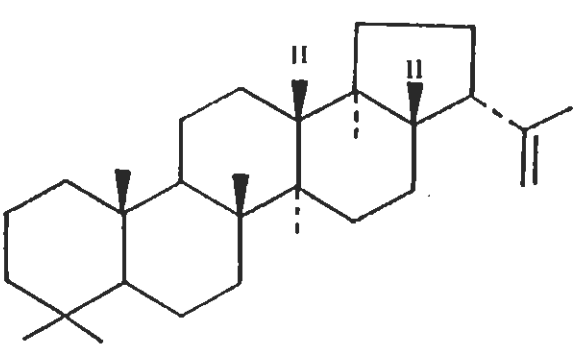


(60)









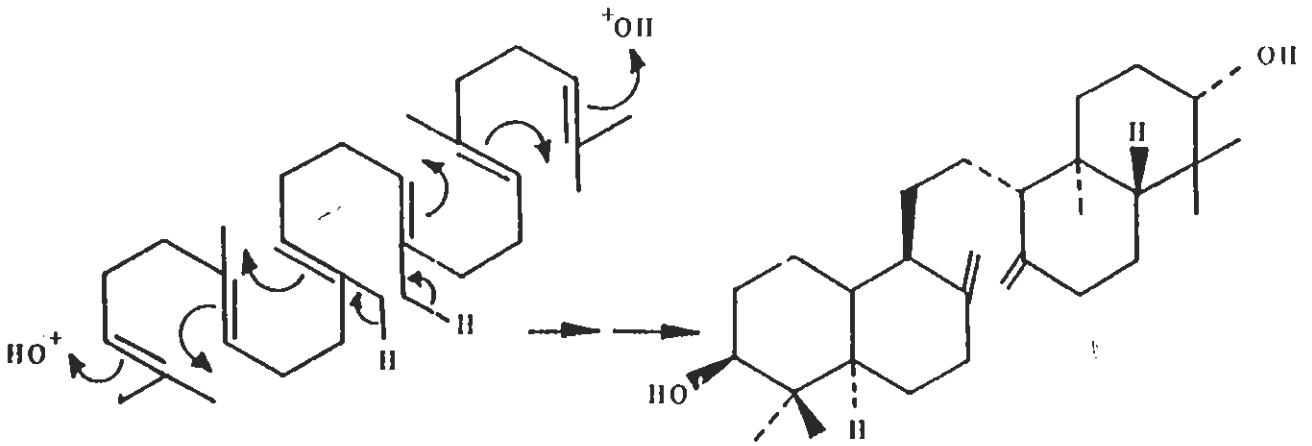
Tetrahymanol (61)

Diploptene (62)

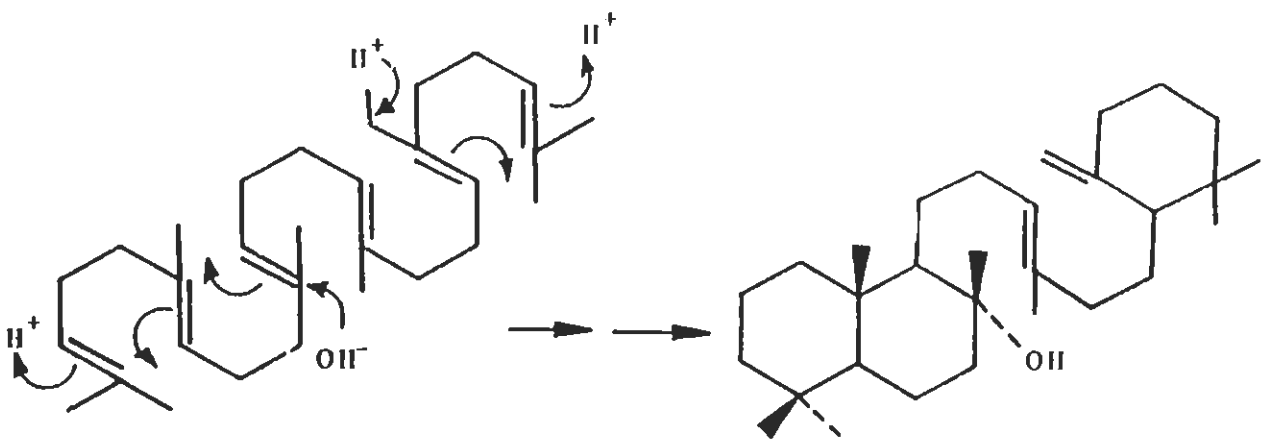
Scheme-18

CYCLIZATION (OXIDATIVE AND NON-OXIDATIVE) INDEPENDENTLY INVOLVING BOTH THE TERMINAL DOUBLE BONDS OF SQUALENE.

The biosyntheses of terpenes such as onocerin (**63**) [37] and embrein (**64**) [38] are explained by admitting two independent electrophilic attacks at both ends of the squalene molecule. The two attacks are both oxidative in the onocerin molecule but in ambrein; it is not known whether they are simultaneous or not (Scheme-19).



Onocerin (63)



Ambrein (64)

Scheme-19

THE DI- AND TRI-TERPENOIDS OF GENUS EUPHORBIA

Euphorbiaceae is one of the largest family of the phylum Anthophyta with 300 genera and 5000 species [39]. It owes its name to king Juba II of Mauritania (25 B.C) who discovered a succulent plant and named it Euphorbia in honour of his physician, Euphorbos [40]. Among various genera of Euphorbiaceae only 24 are found in Pakistan out of which 11 are not native [38]. The largest genera of Euphorbiaceae is Euphorbia which comprises well over 2000 species occurring in the form of laticiferous herbs, shrubs and small trees, inhabiting the tropical and temperate zones of Asia and other parts of the world.

For centuries, plants and plant materials of species of the family Euphorbiaceae have been known to be poisonous to human beings. Often they are held responsible for the poisoning of live stock and are used as consituents of arrow poisons. However, multidisciplinary pharmacological screenings carried out in recent times have shown some of them to be quite useful for the treatment of a variety of ailments like cancer, rheumatism, neuralgia, asthma and bacterial infections [41]. Quite a number of species are also used in argriculture, as resources for certain technologies and as ornamental plants in horticulture. The many fold uses of Euphorbia species have created wide interest in their phytochemistry and a voluminous work on the isolation and structural elucidation of different types of chemical constituents has so far been published.

Chemical sutides in Euphorbia species started as far back as 1834 when Rose and Fluckiger [42-43] obtained a crystalline compound euphorbone from the latices of some of its species. In 1928 Bauer and Schenkel [44] obtained an unsaturated alcohol from euphorbone. Later, Bauer and Schroeder [45] found this alcohol to be a mixture which through repeated fractional crystallization yielded a uniform product,

α-euphorbol (euphorbol) $C_{31}H_{52}O$, m.p. 127-28°, $[\alpha]_D$-2°, along with a low melting alcohol (89-90°), the purity of which was doubtful. Subsequently a large number of chemical constituents, including terpenoids, steroids and flavonoids, were isolated from Euphorbia species. Since di- and tri-terpenes form the subject matter of this part of the thesis, a comprehensive review of those isolated from various Euphorbia species is presented in the following Tables I and II.

Table-1: List of diterpenoids isolated from genus Euphorbia

S.No.	Name	Molecular formula	Molecular wt.	Name of species	References
1.	7-Acetoxy ingenol-5,20-diacetate-3-angelate	$C_{31}H_{40}O_9$	556	E.kam.	46
2.	15-O-Acetyl-3-O-benzoyl-characiol-5,6 -oxide	$C_{29}H_{36}O_7$	496	E.ch.	47
3.	15-Acetyl-3-benzoyl-jolkinol-5,6 -oxide	$C_{29}H_{36}O_6$	480	E.ch.	48
4.	3-O-Acetyl-16-O-benzoyl-20-O-[(Z)-2-methyl-2-butenoyl]-16-hydroxy-ingenol.	$C_{34}H_{40}O_9$	592	E.can.	49
5.	20-O-Acetyl-3-O-(Z,E, 2E) decadienoyl-ingenol	$C_{34}H_{48}O_7$	568	E.bl.	50
6.	20-Acetyl-ingenol decadienoate	$C_{32}H_{44}O_7$	540	E.Bro.	231
7.	20-O-Acetyl-3-O-(2E,4Z)-2,4,6-decatrienoylingenol	$C_{32}H_{42}O_7$	538	E.bl.	50
8.	15-O-Acetyl-5β-hydroxy-isocharaciol-3-benzoate	$C_{29}H_{36}O_7$	496	E.ch.	47
9.	15-O-Acetyl-5β-hydroxy-isocharaciol-3-isobutyrate	$C_{26}H_{38}O_7$	462	E.ch.	47
10.	15-O-Acetyl-5β-hydroxy-isocharaciol-3-tigliate	$C_{27}H_{38}O_7$	474	E.ch.	47
11.	20-Acetyl-ingenol-3-angelate.	$C_{27}H_{36}O_7$	472	E.kam.	51

12.	20-Acetyl-ingenol-3-decadienoate	$C_{32}H_{44}O_7$	540	E.bro.	52
13.	20-Acetyl-ingenol-3-octenoate	$C_{30}H_{42}O_7$	514	E.kam.	51
14.	β-Acetyl-ingol-12-tigliate	$C_{27}H_{38}O_6$	490	E.kam.	53
15.	15-Acetyl-3-isobutyryl-jolkinol-5, 6 -oxide	$C_{26}H_{41}O_6$	446	E.ch.	48
16.	2-Acetyl-3-isobutyryl-15-nicotinoyl-2-hydroxyjolkinol-5,6 -oxide	$C_{32}H_{41}NO_8$	567	E.ch.	48
17.	3-O-Acetyl-20-O[(Z)-2-methyl-2-butenoyl]-ingenol	$C_{27}H_{36}O_7$	472	E.can.	49
18.	15-Acetyl-3-nicotinoyl-jolkinol-5,6-oxide	$C_{28}H_{35}NO_6$	481	E.ch.	48
19.	15-O-Acetyl-3-O-propionyl-characiol	$C_{25}H_{36}O_6$	432	E.ch.	47
20.	15-Acetyl-3-propionyl-jolkinol-5, 6-oxide	$C_{25}H_{36}O_6$	432	E.ch.	48
21.	20-0-Acetyl-resiniferonol-9,13,14-orthophenylacetate	$C_{30}H_{34}O_7$	506	E.po,E.tir.	54,55,56
22.	15-0-Acetyl-3-O-tigloyl-characiol-5,6-oxide	$C_{27}H_{38}O_7$	474	E.ch.	47
23.	15-Acetyl-3-tigloyl-jolkinol-5, 6-oxide	$C_{27}H_{38}O_6$	458	E.ch.	48
24.	3-Angelyl-ingenol	$C_{25}H_{34}O_6$	430	E.pa.	57
25.	3-Angelyl-20-deoxy-ingenol	$C_{25}H_{34}O_5$	414	E.pa.	57

26.	Caudicifoline	$C_{20}H_{26}O_4$	330	E.cad,E.n.	58,59
27.	3-0-(2E,4Z)-Decadienoylingenol	$C_{30}H_{42}O_6$	498	E.es.	60
28.	3-(2,4,6-Decatrienoate)-16-angelyl-ingenol	$C_{35}H_{46}O_8$	594	E.in.	61
29.	3-(2,4,6-Decatrienoate)-ingenol	$C_{30}H_{40}O_6$	496	E.In,E.es.	60,61
30.	3-O-(2E,4Z)-2,4,6-Decatrienoyl-20-deoxy-ingenol.	$C_{30}H_{40}O_5$	480	E.bi.	50
31.	5-O-(2E,4Z)-2,4,6-Decatrienoyl-20-deoxy-ingenol.	$C_{30}H_{40}O_5$	480	E.bi.	50
32.	3-0-(n-Deca-2,4,6-trienoyl)-16-0-[(Z)-2-methyl-2-butenoyl]-16-hydroxy-ingenol	$C_{35}H_{46}O_8$	594	E.herm.	63
33.	12-Deoxy-16-hydroxy-phorbol-13-angelate-16-isobutyrate	$C_{29}H_{40}O_8$	516	E.co.	64
34.	12-Deoxy-16-hydroxy-phorbol-13-angelate-16-isobutyrate-20-acetate.	$C_{31}H_{42}O_9$	558	E.co.	64
35.	5-Deoxy-ingenol	$C_{20}H_{28}O_4$	332	E.m,E.bi.	65
36.	20-Deoxy-ingenol-3-0-angelate	$C_{25}H_{34}O_5$	414	E.pep,E.pa.	57,66
37.	5-Deoxy-ingenol-3,20-diacetate	$C_{24}H_{32}O_6$	416	E.m.	65
38.	12-Deoxy-phorbol	$C_{20}H_{38}O_5$	348	E.tr.	116
39.	20-Deoxy-phorbol-13-0[p-acetoxy phenylacetate]-20-acetate.	$C_{32}H_{38}O_9$	566	E.po.	54,55,56
40.	12-Deoxy-phorbol-13-angelate	$C_{25}H_{34}O_6$	430	E.po,E.u,E.he,E.tir.	67,68,69

41.	12-Deoxy-phorbol-13-angelate-20-acetate.	$C_{27}H_{36}O_7$	472	E.r,E.u, E.he,E.po.	67,68,70
42.	12-Deoxy-4β-OH-phorbol-13-20-diacetate	$C_{24}H_{32}O_7$	432	E.tr.	116
43.	12-Deoxy-phorbol-13,20-diacetate	$C_{24}H_{32}O_7$	432	E.mi.E.tr, E.mi,	71,72,74,116
44.	12-Deoxy-4β-OH-phorbol-13-dodecanoate-20-acetate.	$C_{34}H_{52}O_7$	572	E.coer,E.fo, E.th,E.he,E.h.	72,73,74,75
45.	12-Deoxy-phorbol-13-isobutyrate.	$C_{24}H_{34}O_6$	418	E.po,E.tr.	67,69,76,77,72
46.	12-Deoxy-phorbol-13-isobutyrate-20-acetate.	$C_{26}H_{36}O_7$	460	E.r,E.tir,E.tri, E.p.	56,67,68,70
47.	12-Deoxy-phorbol-13-(2-methyl butyrate)	$C_{25}H_{36}O_6$	432	E.tri,E.po,E.u, E.tr.	67,76,77
48.	12-Deoxy-phorbol-13-(2-methyl butyrate) -20-acetate.	$C_{27}H_{38}O_7$	474	E.po,E.u,E.l, E.lac.	67,69,72
49.	12-Deoxy-4β-OH-phorbol-13-octa noate-20-acetate.	$C_{30}H_{42}O_7$	514	E.poly.	73
50.	4-Deoxy-phorbol-12-octa-2,3-dienoate-13,20-diacetate	$C_{32}H_{42}O_8$	554	E.bi.	78
51.	4-Deoxy-phorbol-12-octa-2,3-dienoate-13-isobutyrate-20-acetate	$C_{34}H_{46}O_8$	582	E.bi.	78
52.	4-Deoxy-phorbol-12-octa-2,3-dienoate-13-propanoate-20-acetate	$C_{33}H_{44}O_8$	568	E.bi.	78

53.	12-Deoxy-phorbol-13-phenyl acetate-20-acetate	$C_{30}H_{36}O_7$	508	E.r,E.tlr,E.he, E.th,E.tr.	67,69,72,75,79,74,70
54.	12-Deoxy-phorbol-13-0-phenyl acetate-16-0-methyl butyrate-20-acetate	$C_{36}H_{40}O_9$	608	E.tlr.	55,80
55.	12-Deoxy-phorbol-13-tetradecanoate	$C_{34}H_{54}O_6$	558	E.tr.	77
56.	12-Deoxy-phorbol-13-tiglate	$C_{25}H_{34}O_6$	430	E.tr.	76,77
57.	15,17-Di-0-Acetyl-3-0-cinnamoyl--17-hydroxy-jolkinol.	$C_{33}H_{40}O_7$	548	E.l.	81
58.	3,7-Diacetyl-ingol-12-tigliate	$C_{29}H_{40}O_9$	532	E.kam.	53,106
59.	8,7-Diacetyl-ingol-12-tigliate	$C_{29}H_{40}O_9$	532	E.kam.	53
60.	3,12-Diacetyl-ingol-7-tigliate	$C_{29}H_{40}O_9$	532	E.kam.	83
61.	3,12-Di-0-acetyl-ingol-8-tigliat	e	$C_{29}H_{40}O_9$	532	E.lac.84
62.	12,20-Dideoxy-phorbol-13-angelate	$C_{25}H_{34}O_5$	414	E.r.	85
63.	12,20-Dideoxy-phorbol-13-isobutyrate	$C_{24}H_{34}O_5$	402	E.r.	85
64.	12-Deoxy-phorbol-13-isobutyrate	$C_{24}H_{34}O_6$	418	E.tr.	77
65.	3-0-(2,4,6,8-Dodecatetraenoyl)-ingenol	$C_{32}H_{42}O_6$	522	E.es.	60
66.	6,20-Epoxy-lathyrol	$C_{20}H_{32}O_4$	336	E.l.	88,87
67.	6,20-Epoxy-lathyrol-3-phenyl acetate-15,5-diacetate	$C_{32}H_{38}O_7$	534	E.l.	88
68.	Ester L_3	$C_{30}H_{38}O_7$	510	E.l.	109

69.	Esulone A	$C_{38}H_{42}O_{12}$	690	E.es.	89
70.	Esulone B	$C_{40}H_{44}O_{12}$	732	E.es.	89
71.	Euphoheline A	$C_{33}H_{44}O_{11}$	616	E.he.	90
72.	Euphoheline B	$C_{31}H_{42}O_{10}$	574	E.he.	90
73.	Euphoheline C	$C_{40}H_{48}O_{12}$	720	E.he.	90
74.	Euphoheline D	$C_{31}H_{38}O_{10}$	570	E.he.	90
75.	Euphoheline E	$C_{31}H_{40}O_{10}$	572	E.he.	90
76.	Euphorbia factor RL14	$C_{28}H_{32}O_6$	464	E.r.	85
77.	Euphorbiasteroid	$C_{32}H_{40}O_8$	552	E.l.	87
78.	Euphornin	$C_{33}H_{44}O_9$	584	E.mad.	91
79.	Euphornin A	$C_{31}H_{42}O_8$	542	E.he.	92
80.	Euphornin B	$C_{31}H_{42}O_8$	542	E.he.	92
81.	Euphornin C	$C_{31}H_{48}O_8$	548	E.he.	92
82.	Euphoscopin A	$C_{31}H_{40}O_8$	540	E.he.	93
83.	Euphoscopin B	$C_{33}H_{42}O_9$	582	E.he.	93
84.	Helioscopinolide A	$C_{20}H_{28}O_3$	316	E.he.	94
85.	Helioscopinolide B	$C_{20}H_{28}O_3$	316	E.he.	94
86.	Helioscopinolide C	$C_{20}H_{26}O_3$	314	E.he.	94
87.	20-Hexadecanoate-ingenol	$C_{36}H_{58}O_6$	586	E.in.	61
88.	3-Hexadecanoate-ingenol	$C_{36}H_{58}O_6$	586	E.in,E.l.	61,95
89.	3-Hexanoyl-20-deoxy-ingenol	$C_{26}H_{38}O_5$	430	E.pa.	57
90.	16-Hydroxy-12-deoxy-phorbol-13-(2-methyl 2-butenoate)-16-	$C_{29}H_{40}O_8$	516	E.co.	57

	isobutanoate				
91.	16-Hydroxy-12-deoxy-phorbol-13-(2-methyl 2-butenoate)-16-isobuta-noate-20-acetate.	$C_{31}H_{42}O_9$	558	E.co.	96
92.	16-Hydroxy-ingenol-3,5,16,20-tetraacetate	$C_{28}H_{36}O_{10}$	532	E.lac.	84.
93.	16-Hydroxy pseudjilkinolide B	$C_{20}H_{28}O_5$	348	E.pa.	97
94.	Ingenol	$C_{20}H_{28}O_5$	348	E.l,E.de,E.seg. E.m,E.bl.	65,98,62,82
95.	Ingenol-3-decanoate	$C_{30}H_{46}O_6$	502	E.kam.	51
96.	Ingenol-3,20-dibenzoate	$C_{34}H_{36}O_7$	556	E.es.	95
97.	Ingenol-3,20-di-isobutyrate	$C_{28}H_{40}O_7$	488	E.cot.	100
98.	Ingenol-3-dodecanoate	$C_{32}H_{50}O_6$	530	E.kam.	51
99.	Ingenol-6-dodecanoate	$C_{32}H_{50}O_6$	530	E.es.	101
100.	Ingenol-3-hexadecanoate	$C_{36}H_{58}O_6$	586	E.l.	98
101.	Ingenol-5-hexadienoate	$C_{26}H_{34}O_6$	442	E.kam.	51
102.	Ingenol-20-isobutyrate	$C_{24}H_{34}O_6$	418	E.cot.	100
103.	Ingenol-12-octenoate	$C_{28}H_{42}O_6$	474	E.pep.	66
104.	Ingenol-5-octenoate	$C_{28}H_{42}O_6$	474	E.kam.	51
105.	Ingenol-3-plamitate	$C_{36}H_{58}O_6$	586	E.ser.	102
106.	Ingenol-3-propanoate-20-isobutyrate	$C_{27}H_{38}O_7$	474	E.cot.	100
107.	Ingenol-3-propanoate-20-(2-methyl	$C_{28}H_{40}O_7$	488	E.cot.	100

	butanoate)				
108.	Ingenol-3-propanoate-4,5, 20-triacetate	$C_{31}H_{42}O_{11}$	590	E.po.	99
109.	Ingenol-6-tetradeca-2,4,6,8,10-pentenoate.	$C_{34}H_{44}O_8$	548	E.es.	101
110.	Ingenol-3-tetradeca-2,4,6,8,10-pentenoate.	$C_{34}H_{44}O_8$	548	E.jo,E.l.	103,104
111.	Ingenol-3,5,20-triacetate	$C_{26}H_{34}O_8$	474	E.m,E.ser, E.seg,E.es,E.l, E.lac,E.h.	65,98,75,105,106
112.	Ingol-7-(p-methoxy phenylacetate)-3,8,12-triacetae	$C_{34}H_{42}O_{10}$	610	E.r.	70
113.	Ingol-7-phenylacetate-3,8,12-triacetate.	$C_{34}H_{42}O_{10}$	610	E.r.	70
114.	Ingol-3,7,8,12-tetraacetate.	$C_{28}H_{38}O_{10}$	534	E.kam.	53
115.	Ingol-3,8,12-triacetate-7-angelate	$C_{31}H_{42}O_{10}$	574	E.kam.	107
116.	Ingol-3,7,12-triacetate-8-nicotinate	$C_{32}H_{39}NO_{10}$	597	E.in.	108
117.	Ingol-3,8,12-triacetate-7-tiglate	$C_{31}H_{42}O_{10}$	574	E.kam.	107
118.	12-0-Isobutyl-phorbol-13-acetate-20-angelate.	$C_{31}H_{42}O_9$	558	E.coer,E.far.	110
119.	Jolkinol A.	$C_{29}H_{36}O_6$	480	E.l.	111
120.	Jolkinol A'.	$C_{26}H_{32}O_6$	440	E.l.	111

121.	Jolkinolide A	$C_{20}H_{26}O_3$	314	E.jo,E.n.	58,59,1
122.	Jolkinolide B	$C_{20}H_{26}O_4$	330	E.jo.	112
123.	Jolkinolide C	$C_{19}H_{26}O_3$	302	E.jo.	113
124.	Jolkinolide D	$C_{19}H_{26}O_3$	302	E.jo.	113
125.	Jolkinolide E	$C_{19}H_{28}O_2$	288	E.jo.	113
126.	3-0-[(Z)-2-Methyl-2-butenoyl] 20-0-acetyl-ingenol	$C_{27}H_{36}O_7$	472	E.herm.	63
127.	3-O-[(Z)-2-Methyl-2-butenoyl]-16-O-acetyl-20-deoxy-16-hydroxy-ingenol	$C_{27}H_{36}O_7$	472	E.herm.	63
128.	3-0-[(Z)-2-Methyl-2-butenoyl]-16-0-benzoyl-16-hydroxy-ingenol	$C_{32}H_{38}O_8$	550	E.can.	49
129.	3-0-[(Z)-2-Methyl-2-butenoyl]-16,20-diacetyl-16-hydroxy-ingenol	$C_{29}H_{38}O_9$	556	E.herm.	63
130.	3-0-[(Z)-2-Methyl-2-butenoyl]-5, 16,20-0-triacetyl-16-hydroxy-ingenol	$C_{31}H_{40}O_{10}$	572	E.herm.	63
131.	8-0-Methyl-ingol-12-acetate-7-angelate.	$C_{28}H_{40}O_8$	504	E.kam.	107
132.	8-0-Methyl-ingol-3,12-diacetate-7-angelate	$C_{30}H_{42}O_9$	546	E.kam.	107
133.	8-0-Methyl-ingol-3,12-diacetate-7-benzoate	$C_{32}H_{40}O_6$	568	E.kam.	107
134.	8-0-Methyl-ingol-3,12-diacetate-7-tiglate	$C_{30}H_{42}O_9$	546	E.kam,E.her.	119

135.	Milliamine C	$C_{43}H_{42}N_3O_9$	749	E.mi.	103
136.	Milliamine H	$C_{37}H_{40}O_9N_2$	656	E.mi.	114
137.	Milliamine 1	$C_{36}H_{40}O_{10}N$	645	E.mi.	114
138.	12-0-(2Z,4E) Octadienoyl-4-deoxyphorbol-13-acetate.	$C_{30}H_{40}O_7$	512	E.tir.	115
139.	12-0-(2Z,4E) Octadienoyl-4-deoxy phorbol-13,20-diacetate.	$C_{32}H_{42}O_8$	554	E.bro.	52
140.	12-0-(2Z,4E) Octadienoyl-phorbol 13,20-diacetate	$C_{31}H_{42}O_9$	570	E.bro.	52
141.	Phorbol	$C_{20}H_{28}O_8$	364	E.fran.	117
142.	Proresiniferatoxin	$C_{37}H_{42}O_{10}$	646	E.r.	85
143.	Pseudojolkinolide A	$C_{20}H_{26}O_3$	314	E.pa.	97
144.	Pseudojolkinolide B	$C_{20}H_{26}O_4$	330	E.pa.	97
145.	Resiniferanol	$C_{20}H_{28}O_6$	364	E.r.	85
146.	Resiniferatoxin	$C_{37}H_{40}O_9$	628	E.r,E.u.	85
147.	3-Tetradecanoate-ingenol-5,20-diacetate.	$C_{38}H_{58}O_8$	642	E.bro.	52
148.	20-Tetradecanoate-ingenol-3,5-diacetate.	$C_{38}H_{58}O_8$	642	E.bro.	52
149.	5-Tetradecanoate-ingenol-3,20-diacetate.	$C_{38}H_{58}O_8$	642	E.bro.	52
150.	Tirucallcine	$C_{27}H_{38}O_9$	506	E.tir.	232
151.	3,7,12-Triacetyl-8-angelyl-ingol	$C_{31}H_{42}O_{10}$	534	E.kam.	118

152.	3,7,12-0-Triacetyl-8-0-benzoyl-18-hydroxy-ingol.	$C_{32}H_{40}O_{10}$	584	E.herm.	119
153.	5,15,17-Tri-0-acetyl-3-0-benzoyl-17-hydroxy-isolathyrol	$C_{33}H_{44}O_9$	580	E.l.	81
154.	3,7,12-Triacetyl-8-benzoyl-ingol	$C_{33}H_{40}O_{10}$	596	E.kam.	118
155.	3,7,8-Triacetyl-ingol-12-tigliate	$C_{31}H_{42}O_{10}$	574	E.kam.	82,106
156.	2,5,8,15-0-Triacetyl-nicotinoyl-2,5,8-trihydroxy-isocharaciol-3-benzoate.	$C_{39}H_{43}NO_{12}$	717	E.ch.	47
157.	2,5,8,15-0-Triacetyl-nicotinoyl-2,5,8-trihydroxy-isocharaciol-3-tigliate.	$C_{37}H_{45}NO_{12}$	695	E.ch.	47
158.	3,7,12-Triacetyl-8-isovaleryl-ingol	$C_{31}H_{44}O_{10}$	576	E.tir.	230
159.	3,7,12-Triacetyl-8-tiglyl-ingol	$C_{31}H_{42}O_{10}$	534	E.kam.	118
160.	Triaculetin	$C_{20}H_{28}O_2$	300	E.tria.	120

Table-2 : List of triterpenoids isolated from genus Euphorbia

S.No.	Name	m.p. (°C)	Optical rotation ($CHCl_3$)	Molecular formula	Name of species	References
1.	α-Amyrin	186°	+91.6° (Benzene)	$C_{30}H_{50}O$	E.p,E.ro,E.tir,E.tri.	121-125
2.	β-Amyrin	197-97.5°	+99.8° (Benzene)	$C_{30}H_{52}O$	E.p,E.a,E.b, E.cy,E.f,E.h, E.m,E.pa,E.r, E.t,E.tri,E.par, E.trel,E.fer,E.h.	121-123,126-139
3.	β-Amyrin acetate	232.4°	+78.6° (Benzene)	$C_{32}H_{52}O$	E.e,E.m,E.pep, E.fer,E.h,E.mi.	75,136,139,140-144
4.	δ-Amyrinone	198°	-12°	$C_{30}H_{48}O$	E.par.	137
5.	Balatol (Resiniferol)	118.2°	-	$C_{30}H_{50}O$	E.r,E.po.	134-135,140-141,145
6.	Betulin (Pyridine)	251-52°	+19.96°	$C_{30}H_{50}O$	E.pa,E.l.	132-135,140,141,145,146
7.	Betulinic acid	275-78°	+79°	$C_{30}H_{48}O_3$	E.d.	147
8.	Butyro Spermol	111-13°	-12°	$C_{30}H_{50}O$	E.s,E.bi.	239
9.	Butyro Spermol acetate	146.5-47.5°	+11°	$C_{32}H_{52}O$	E.rj.	150
10.	Campesterol	157-58°	-33°	$C_{28}H_{48}O$	E.p,E.e,E.i, E.pa,E.pep,	124,136,140,151-153

					Ero,E.spl.	
11.	Citrostadienol	166-67°	-	$C_{30}H_{50}O$	E.spl.	154
12.	Corollatadiol	193-95°	-	$C_{31}H_{52}O_2$	E.c.	155
13.	Cycloartanol	101-102°	+45°	$C_{30}H_{52}O$	E.trl,Etir.	123,233
14.	Cycloartenol	115°	+54°	$C_{30}H_{50}O$	E.mo,E.rj,E.pa, E.a,E.b,E.es, E.lat,E.se,E.tir, E.pet,E.tri,E.ca, E.bi,E.th,E.n.	74,79,122,123,125, 135,141,143,144, 149,150,152,156 -162
15.	Cycloart-23-en-3β-ol	112-14°	+49.2°	$C_{30}H_{50}O$	E.pol.	163
16.	Cycloart-25-en-3β-ol	85°	+23°	$C_{30}H_{50}O$	E.ni.	164
17.	Cycloartenol acetate	118°	-	$C_{32}H_{52}O$	E.pal,E.bi.	149,165
18.	Cycloart-23-ene-3β,25-diol	200-204°	+38°	$C_{30}H_{50}O_2$	E.cy,E.tir.	128
19.	Cycloartenone	109°	+240°	$C_{30}H_{48}O$	E.b.	127
20.	Cyclocaducinol	114-16°	+31.8°	$C_{30}H_{50}O$	E.ca.	234
21.	Cycloeucalenol	138-40°	+45°	$C_{30}H_{50}O$	E.ro,E.pa,E.tir.	144,166,238
22.	Cycloeuphordenol	105-106°	+39°	$C_{30}H_{50}O$	E.tir.	235

23.	Cycloeuphornol	95°	+46°	$C_{31}H_{50}O$	E.tir.	167
24.	Cyclolaudenol	125°	+46°	$C_{31}H_{52}O$	E.ca,E.ni.	161
25.	3-Epi cyclolaudenol	140°	-10°	$C_{31}H_{52}O$	E.cad.	161
26.	Cycloroylenol	103-105°	+32.2°	$C_{30}H_{50}O$	E.ro.	168
27.	Cyclotrilueanenol	94°	42.3°	$C_{31}H_{52}O$	E.tir.	226
28.	(E,Z)2,4-Decadienoyl-lanosterol.	viscous oil	-	$C_{40}H_{64}O_2$	E.bi.	149
29.	(E,Z) 2,4,6-Decatrienoyl-lanosterol.	viscous oil	57°	$C_{40}H_{62}O_2$	E.bi.	149
30.	β-Dihydrofucusterol	113-14°	-	$C_{29}H_{50}O$	E.he.	169
31.	Epigermanicol	220	-	$C_{30}H_{50}O$	E.ca.	170
32.	Epifriedelinol	281-82°	+8.7°	$C_{30}H_{52}O$	E.a.	171
33.	Epiteraxerol	-	-	$C_{30}H_{50}O$	E.ro,E.th.	74,172
34.	Euphol	115°	+32°	$C_{30}H_{50}O$	E.tir,E.a,E.cy, E.mo,E.ro,E.ad, E.cal,E.can,E.cand, E.car,E.coer,E.ha, E.ja,E.n,E.r,E.wal, E.ka,E.tri,E.ru,E.cad.	123,126,128,141,143, 166,173-185,236

35.	β-Euphol	125°	-	$C_{30}H_{50}O$	E.r.	181
36.	Euphorbinol	111-12°	+54.5°	$C_{31}H_{52}O$	E.tir.	186
37.	Euphorbol	127-28°	+0°	$C_{31}H_{52}O$	E.a,E.cal,E.can, E.cand,E.coer, E.in,E.ja,E.wal, E.d,E.tin,E.r, E.tr,E.th,E.mi, E.lac,E.n.	74,79,126,174,175, 177,179,182,142,143, 187-193
38.	β-Euphorbol	89-90°	-	$C_{31}H_{52}O$	E.cand.	175
39.	δ-Euphorbol	114-15°	+34°	$C_{31}H_{52}O$	E.f,E.tin.	129,194
40.	Euphorbol-hexacosonate	110-11°	-	$C_{57}H_{102}O_2$	E.ja,E.mi,E.tin, E.n,E.h.	74,79,142,143,179
41.	Euphorcinol	189-90°	+25°	$C_{30}H_{50}O$	E.tir.	227
42.	Euphorginol	168-70°	22.35°	$C_{30}H_{50}O$	E.tir.	228
43.	Euphoron	119°	+57.25°	$C_{30}H_{48}O$	E.tir.	195
44.	Euphosterol	274-75°	-	$C_{25}H_{40}O$	E.p.	196
45.	Friedelan-3α-ol	302-304°	+18°	$C_{30}H_{52}O$	E.a,E.n,E.tri.	122,197,198
46.	Friedelan-3β-ol	284°	+13.6°	$C_{30}H_{52}O$	E.a,E.n,E.tri.	122,197,198

47.	Friedelin	262-63°	-29.4°	$C_{30}H_{50}O$	E.h,E.tri.	130,122
48.	Germanicol	176-77°	+5.8°	$C_{30}H_{50}O$	E.b.	199
49.	Germanicone	185-86°	-	$C_{32}H_{48}O$	E.b.	127
50.	Glut-5(6)-en-3α-ol	203-205°	+61°	$C_{30}H_{50}O$	E.cy.	128
51.	Glut-5(6)-en-3β-ol	210.5-11.5°	+64°	$C_{30}H_{50}O$	E.cy,E.tir.	128,200,237
52.	Glut-5-en-3-one	245-46°	+31°	$C_{30}H_{48}O$	E.cy,E.s.	128,148
53.	Glut-5(10) -en-1-one	312-14°	+50° (Ethanol)	$C_{30}H_{48}O$	E.n.	201
54.	Glut-5-en-3β-yl-acetate	188-92°	+76°	$C_{32}H_{52}O_2$	E.ro,E.mac.	202,229
55.	Handianol	111-12°	-	$C_{30}H_{50}O$	E.ha,E.ap,E.her, E.o,E.at,E.br.	178,203-205
56.	Hopenol-B	251-53°	+158°	$C_{30}H_{50}O$	E.su.	206
57.	Lanosterol	140-41°	+58°	$C_{30}H_{50}O$	E.lat,E.b,E.rj, E.tir,E.pa,E.pep.	136,144,159,199,205
58.	Lanosteryl acetate	136°	-	$-C_{32}H_{52}O_2$	E.bl.	149
59.	Lupenone	170°	+63.5°	$C_{30}H_{48}O$	E.b,E.ph,E.w.	127,207,208
60.	Lupeol	215-16°	+26.4°	$C_{30}H_{50}O$	E.b,E.pa,E.mo,	122,123,127,132,141,

					E.al,E.es,E.se, E.ph,E.tri,E.g, E.d.	158,165,160,207, 209-211
61.	Lupeol acetate	217-18°	+47.5°	$C_{32}H_{52}O_2$	E.w,E.g.	208,210
62.	Maculatol	197-98°		-$C_{30}H_{50}O$	E.mac.	212
63.	4-Methyl-cycloart-22-ene-3β, 25-diol	-	-	$C_{29}H_{48}O_2$	E.lac.	213
64.	24-Methylene-cycloartanol	162-64°	+36°	$C_{31}H_{52}O$	E.cy,E.e,E.rj, E.la,E.lat,E.tir, E.se,E.es,E.mi, E.pa,E.th,E.pe, E.tlr,E.trl,E.lac, E.cad,E.h,E.bi,E.n.	74,122,128,149,150, 158,159,160,79, 144,143,140,162, 184,192,209,214, 193
65.	Methyl-3-oxoursolate	-	-	$C_{31}H_{48}O_3$	E.ca.	161
66.	Moretenol	236°	+27°	$C_{30}H_{50}O$	E.la.	212
67.	Moretenone	202-204°	+54°	$C_{30}H_{48}O$	E.la,E.par,E.het.	137,138,212
68.	Nerifoliol	130-31°	+20°	$C_{30}H_{50}O$	E.cal,E.n.	180,185
69.	Obtusifoliol	138-40°	+72.1°	$C_{31}H_{52}O$	E.f,E.ech,E.br,	129,149,204,

					E.rj,E.o,E.bi.	205,215
70.	(E,Z)2,4-Octa-dienoyl-cycloartenol	oil	-	$C_{38}H_{60}O_2$	E.bi.	149
71.	(E,Z)2,4-Octa-dienyltenosterol	Viscous oil	-	$C_{38}H_{60}O_2$	E.bi.	149
72.	Olean-13(18) -en-3-one	-	-	$C_{30}H_{48}O$	E.ph.	205
73.	Oleanolic acid	306-308°	+79.5°	$C_{30}H_{48}O_3$	E.pa,E.d.	132,211
74.	Spiosupinanonediol	248-85°	-3.8°	-	E.su.	216
75.	Taraxerol	282-83°	±0°	$C_{30}H_{50}O$	E.h,E.m,E.r, E.l,E.i,E.ad, E.cal,E.a;E.n, E.tir,E.jo,E.p, E.ro,E.th.	130,131,134,146,151, 173,174,197,198, 201,217-221
76.	Taraxerol	267-69°	-11.6°	$C_{30}H_{50}O$	E.tir.	233
77.	Taraxerone	242-44°	+11°	$C_{30}H_{48}O$	E.ph,E.l,E.ad, E.a,E.w,E.jo,E.p, E.jac,E.tri, E.par,E.h.	79,137,143,148,173, 197,207,208,217, 218,222-224
78.	Taraxeryl acetate	304-305°	+9°	$C_{32}H_{52}O_2$	E.ro,E.ph,E.w, E.jo,E.tri,E.tir.	122,202,207,208, 217,210,238

79.	Taraxasterol	225-26°	+95.9°	$C_{30}H_{50}O$	E.w,E.tir,E.g.	210,207,225
80.	Taraxasterol acetate	256-57°	100.5°	$C_{32}H_{52}O_2$	E.g.	210
81.	Tirucallol	133-34.5°	+4.5°	$C_{30}H_{50}O$	E.coer,E.th, E.tir,E.ru.	177,183,220,225
82.	Ursa-9(11),12-dien-3β-ol	150-51°	362.6°	$C_{30}H_{50}O$	E.mac.	229
83.	Ursolic acid	291°	+66° (Ethanol)	$C_{30}H_{48}O_3$	E.pa.	132
84.	Uvaol	222-24°	+70°	$C_{30}H_{50}O_2$	E.pa.	132

The abbreviations in the table stand for the following species:

E.ad = E. adenochlora, E.a = E. antiquorum, E.ap = E. aphyla, E. at = E. atropurpurea, E.b = E. balsimifera, E.bi = E.biglandulosa, E.br = E. bravoana, E.bro = E. broteri, E.cal = E. caltimondos, E. can = E. Canariensis, E. cand = E. candylocarpa, E.ca = E. candellia, E.ch = E.characias, E. car = E. caracasana, E. cad = E. caducifolia, E. coer = E. coerlesecns, E. cot = E. cotinifolia, E.c = E. corollata, E. cy = E. cyparissias, E. co = E. cooperi, E.d = E. dracunculoides, E. de = E. desmondi, E.e = E. ebracteolata, E. ech = E. echinus, E. es = E. esula, E.f = E. falcata, E. fer = E. ferganensis, E. fra = E. franckiana, E. fo = E. fortissime, E. g = E. granulata, E. ha = E. handiensis, E. he = E. helioscopia, E. her = E. hernandez, E. herm = E. hermentiana, E. het = E. heterophylla, E. h = E. hirta, E.i = E. indica, E. in = E. ingens, E. jac = E. Jacquemonti, E. ja = E. jaxartica, E. jo = E. jolkin, E. ka = E. kansui, E. kam = E. kamerunica, E. lac = E. lactea, E. lan = E. lanata, E. la = E. laterifolia, E.l = E. lathyrus, E. lat = E. latzi, E. mad = E. maddeni, E. mac = E. maculatta, E. mi = E. millii, E. mo = E. moilli, E.m. = E.myrisinites, E.n = E. nerifolia, E.nl = nivulia, E.o = E. obtusifolia, E. pal = E. pallasii, E. pa = E. paralias, E. par = E. parvifolora, E. pel = E. pelas, E. pet = E. petiolata, E. ph = E. phsphora, E.pi = E. pilulifera, E. po = E. poisonii, E. pol = E. polygonifolia, E. poly = E. polyacantha, E. pul = E. pulchrima, E.rj = E. regisjubae, E.r = E. resinifera, E. re = E.respetii, E. ro = E. royleana, E. ru = E. ruspolli, E. se = E. segetalis, E.s = E. sikkimensis, E. spl = E. Splendens, E. ser = E. serrata, E. seg = E. seguieriana, E. su = E. supina, E.t = E. teracine, E.th = E. thymifolia, E. tin = E. tinctoria, E. tir = E. tirucalli, E. tr = E. Triangularis, E. tria = E. triaculeata, E.tin = E. tinctoria, E. tri = E. trigona, E.u = E. unispina, E. wal = E. wallichii, E.w = E. watandii.

RESULTS AND DISCUSSION

Euphorbiaceae is a large family with 300 genera and more than 5,000 species [**39**]. Among various genera of Euphorbiaceae only 24 are found in Pakistan out of which 11 are not native [**38**]. Euphorbia is the largest genera of Euphorbiaceae which comprises over 2,000 species occurring in the form of lacticiferous herbs, shrubs, and small trees inhabiting the tropical and temperate zones of Asia and other parts of the world.

The multidisciplinary pharmacological screenings carried out in recent times on plant materials of Euphorbiaceae have shown some of them to be quite useful for the treatment of a variety of ailments like cancer, rheumatism, neuralgia, asthma, and bacterial infections [**41**]. The multifold uses of these species have prompted us to carry out phytochemical studies on Euphorbia clarkeana which is an annual, glabrous, prostrate herb with many branches and is widely distributed in fields, sandy soil and as graden weed in India, Afghanistan and Pakistan [**240**].

A careful sifting of literature sarvey revealed that uptill now no report of chemical work on this plant has appeared. The present work has been undertaken on the freshly collected plant materials from the Karachi District with particular reference to the isolation and structural elucidation of new terpenoids. The techniques employed for the purpose follow the traditional and as well as various forms of adsorption chromatography.

The freshly collected plant material was chopped into small pieces and then extracted thrice with methanol. The combined methanolic extract was evaporated under reduced pressure to afford a gummy residue. This residue was partitioned between hexane and water. The residue recovered from hexane fraction was chromatographed over activated silica gel column and the elution was carried out with solvent gradients of increasing polarities of a mixture of hexane and chloroform.

The eluate obtained from hexane- chloroform in ratio of 7:3 yielded an oily residue which on further purification by preparative layer chromatography in the same solvent system provides 20-acetyl-ingenol-3-decadienoate (**68**). The eluates from hexane-chloroform (6:4 and 5.5:4.5) yielded crystalline residues which on repeated crystallization from hexane-chloroform provided cycloartanol (**66**) and a mixture of triterpenes. The later were resolved through flash column chromatography over silica gel using hexane-chloroform (7:3) as solvent system to yield cycloart-23-ene-3β-25-diol (**67**) and cycloclarkeanol (**65**), respectively. On the other hand the eluates obtained from hexane-chloroform in different ratios (9:1 to 7.5:2.5) yielded three unreported triterpenes namely lupeol (**69**), taraxasterol (**70**) and α-amyrin (**71**). These were identified by comparing with the data available in literature [**241-243**].

STRUCTURE ELUCIDATION OF CYCLOCLARKEANOL

[cycloart-22-ene-3β,20ξ,25-triol] (**65**)

Cycloclarkeanol formed colorless shining needles on repeated crystallization from chloroform/methanol, melted at 190-92^{o} and showed $[\alpha]_D$ 37.84^{o} ($CHCl_3$). It showed molecular ion peak in high resolution mass spectrum (HRMS) at m/z 458.3753 corresponding to the molecular formula $C_{30}H_{50}O_3$ (calcd. 458.3747) and six double bond equivalents in the molecule. The molecular ion peak was also confirmed by field desorption mass spectrometry (FDMS). Cycloclarkeanol gave positive Liebermann-Burchard test and violet coloration with ceric sulfate indicating its triterpenic nature. The IR spectrum of the compound showed absorptions at 3460-3440 (OH group), 3065, 1630 (C=C), 3040 (CH_2 assym. stretching of a cyclopropane ring), 1380 (gem-dimethyl), and 1015 (alkyl cyclopropane). Additional bands between 1180-935 cm^{-1} are fairly in agreement with 3β-OH, 5α structure [244].

The ^{1}H-NMR spectrum of cycloclarkeanol showed signals for olefinic protons (d at δ 5.48, J = 7.05 Hz and m at δ 5.40 Hz), a methine proton attached to the carbon bearing the hydroxyl group (δ 3.26, 1H, dd, $J_{a,a}$ = 10.78 Hz and $J_{a,e}$ = 4.58 Hz) seven-tertiary methyls (singlets at δ 1.39, 0.87, 0.80, 1.33 and 0.95), and a pair of doublets, indicative of cyclopropane ring bearing two non-equivalent hydrogen atoms (δ 0.33, J = 4.26 Hz and δ 0.54, J = 4.20 Hz).

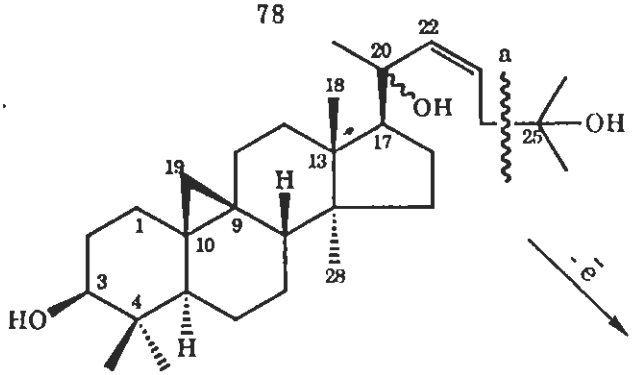
The ^{13}C-NMR spectrum showed 30 carbon atoms; the multiplicities of these were determined by using DEPT experiments [245,246], which revealed the presence of 7 methyl, 10 methylene, and 6 methine carbon atoms. The number of quaternary carbons were determined by substracting these from the broad-band spectrum.

Both the ^{1}H- and ^{13}C-NMR spectra indicated the presence of one secondary and two tertiary hydroxyl groups which was further confirmed by acetylation of **65** to a monoacetate **65a** showing molecular ion peak at m/z 500.3839 and a broad band at 3460cm^{-1} for OH group in the IR spectrum.

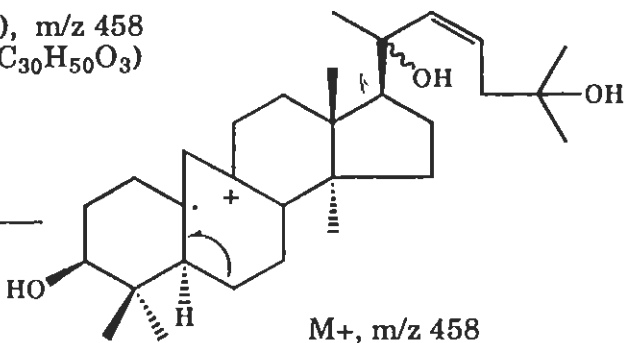
A wealth of structural information was obtained from the mass spectrum of **65** which was characteristic of tetracyclic triterpenes containing the 9,19-cyclo function. The genesis of various fragments could be confirmed by link-scan measurements. The characteristic ion "a" at m/z 318.2520 ($C_{21}H_{34}O_2$) represented the fragmentation induced by a cyclopropane ring in 9,19 cyclosterols and related tetracyclic triterpenes [247,248]. The strain imposed on rign B was relleved by opening of the 9,10-bond followed by cleavage of the 5,6-bond and McLafferty rearrangement. The loss of the side chain from this ion gave another fragment "b" at m/z 175.1461 ($C_{13}H_{19}$). Further ions at m/z 443.3510 ($C_{29}H_{47}O_3$), 44.3675 ($C_{30}H_{48}O_2$), and 425.3528 ($C_{29}H_{45}O_2$) originated by the loss of a methyl radical, water, and methyl plus water, respectively, from the molecular ion.

The fragment at m/z 315.2638 and 175.1461 had same compositions as those reported for cycloartanol [247,248] from which it can be inferred that two of the three hydroxyl groups and the olefinic bond were present in the side chain and the remaining hydroxyl group in ring A/B. The presence of a monounsaturated side chain with two oxygen functions was indicated by the peak at m/z 315.2638 ($C_{22}H_{35}O$) resulting from the direct loss of the side chain from the molecular ion peak at m/z 458.3753. The peak at m/z 399.3251 resulted from allylic cleavage of C 24-25 bond and allowed us to place the double bond at C-22 [249]. A comparison of the chemical shift of the side chain carbon atoms with those of stigmasterol revealed downfield shift of methyl groups at C-20 and C-25 in **65** [250]. Therefore, the tertiary hydroxy groups can safely be assigned to these positions.

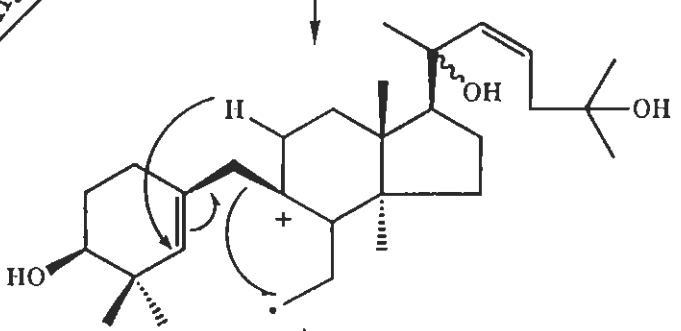
The secondary hydroxyl group was assigned to C-3 on the basis of biogenetic analogy and also by ^{1}H-^{1}H- correlated spectroscopy. The carbinylic proton at δ 3.26 showed cross peaks with two other protons limiting its presence to positions 1 or 3. However, the chemical shifts of C-2, C-4 and the methyl groups attached to it showed close agreement to cycloartanol providing evidence for the presence of hydroxyl group at C-3 rather than C-1. The chemical shifts and coupling constants of the carbinylic proton were in accord with its axial and α-orientation thereby structure **65** can be assigned to cycloclarkeanol. The ^{13}C- and ^{1}H-^{13}C correlated spectra are in complete agreement to the assigned structure. The configuration at C-20 could not be determined in the present work.

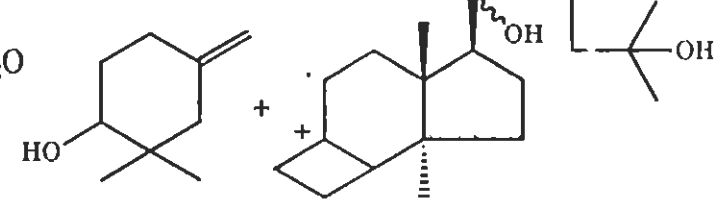


(65), m/z 458
($C_{30}H_{50}O_3$)

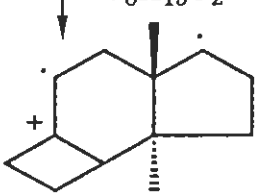


M+, m/z 458
($C_{30}H_{50}O_3$)





m/z 316
($C_{21}H_{34}O_2$)



m/z 175
($C_{13}H_{19}$)

m/z 440
($C_{30}H_{48}O_2$)

m/z 422
($C_{30}H_{46}O$)

m/z 315
($C_{22}H_{35}O$)

m/z 443
($C_{29}H_{47}O_3$)

m/z 297
($C_{22}H_{33}$)

m/z 425
($C_{29}H_{45}O_2$)

m/z 407
($C_{29}H_{43}O$)

m/z 300
($C_{21}H_{32}O$)

- H_2O

- H_2O

- $C_8H_{15}O_2$

- CH_3

- H_2O

- H_2O

- CH_3

- H_2O

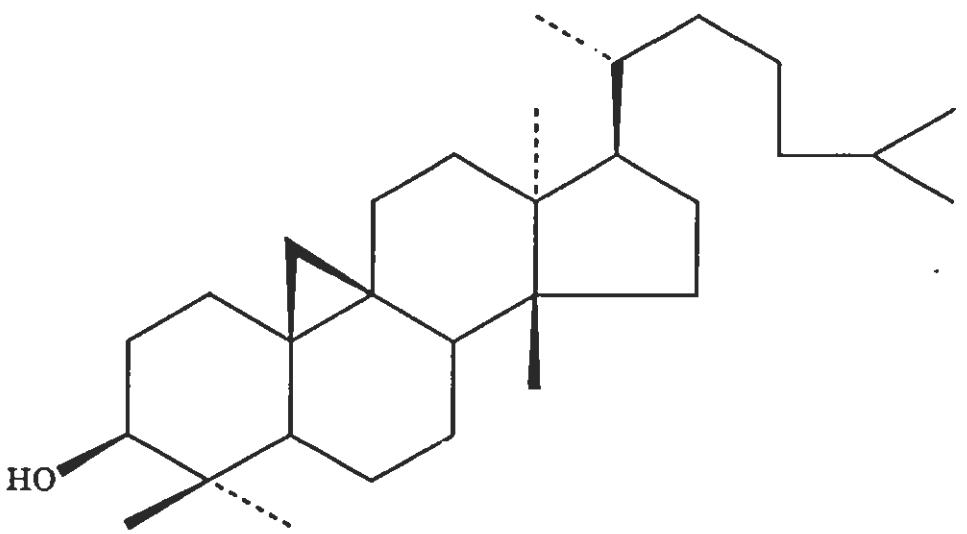
- $C_8H_{15}O_2$

STRUCTURE ELUCIDATION OF CYCLOARTANOL (66)

Cycloartanol formed colorless shining needles from 90% methanol, melted at 100-102^{o} and showed $[\alpha]_D$ 49.7^{o} ($CHCl_3$). Its IR spectrum showed absorption bands for hydroxyl group (3430 cm^{-1}) and cyclopropane ring (3045 cm^{-1}). The HR mass spectrum of **66** afforded the molecular ion peak at m/z 428.4018 corresponding to the molecular formula $C_{30}H_{52}O$ (calcd. 428.4012), suggesting five degree of unsaturation. The mass spectrum showed characteristic fragmentation pattern of cycloartane type triterpenes [247,248].

The ^{1}H-NMR spectrum revealed characteristic double doublet at δ 0.33 and 0.56 (J = 4.0 Hz) for non-equivalent cyclopropyl methylene protons, another pair of doublets at δ 3.27 was typical of carbinylic proton at the usual 3-position in α- and axial orientation. The absence of any other signals at δ 3.27 confirmed the saturated nature of **66**. It further showed the presence of four tertiary [δ : 0.82 (3H), 0.88(3H). 0.96 (6H)] and three secondary [δ 0.90 (6H), 0.87(3H)] methyl groups. The ^{13}C-NMR spectrum of **66** showed signals of 30 carbon atoms; the multiplicity assignments of each of these was determined by DEPT experiments.

On the basis of comparison of physical constants and spectral data with the literature [248,249], the compound isolated was identified as cycloartanol (**66**). Previously it was isolated from polypodium vulgare [248].



(66)

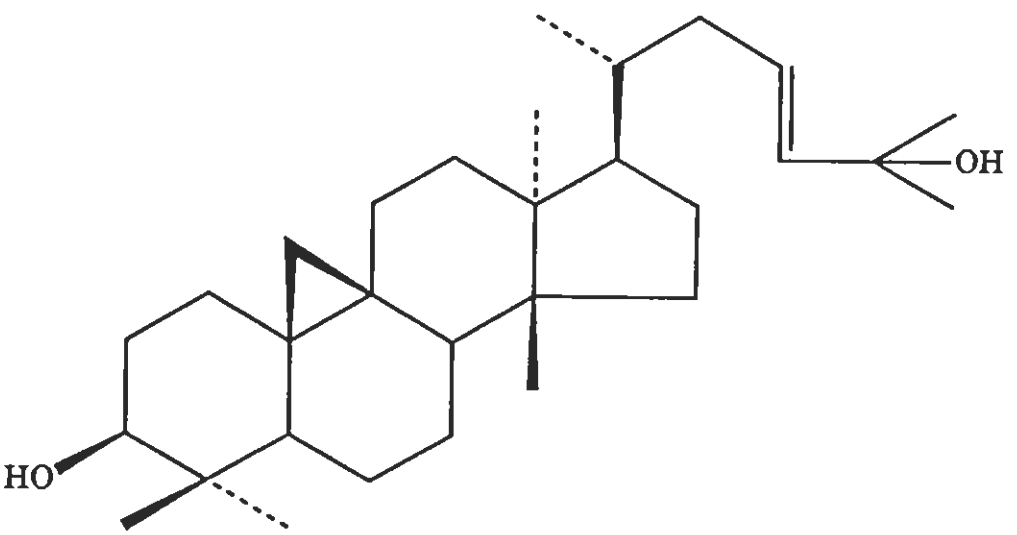
STRUCTURE ELUCIDATION OF CYCLOART-23-ENE-3β-25-DIOL (67)

Cycloart-23-ene-25,3β-diol formed colorless shining needles from acetone, melted at 196-198^{o} and showed $[\alpha]_D$ 33.9^{o} ($CHCl_3$). It showed molecular ion peak at m/z 442.3810 (HR mass spectrometry), corresponding to the molecular formula $C_{30}H_{50}O_2$ (calcd. 442.7280). Its IR spectrum revealed the presence of hydroxyl groups (3590, 3440 cm^{-1}) and cyclopropyl ring (3040 cm^{-1}).

The HR mass spectrum of **67** showed characteristic features of tetracyclic triterpenes of cycloartane series [247,250]. The diagnostic fragment ions appeared at m/z 357.6041 $(C_{25}H_{41}O)^+$, 302.5101 $(C_{21}H_{32}O)^+$, 284.4861 $(C_{21}H_{32}\text{-}H_2O)^+$, 315.5251 $(C_{22}H_{35}O)^+$, 297.5144 $(C_{22}H_{33}\text{-}H_2O)^+$ and 175.2943 $(M\text{-}C_7H_{31}O_2)^+$. The fragment ion peak at m/z 315.5251 resulted by the loss of entire substituent at C-17 and revealed that the second hydroxyl group and double bond were present in the side chain.

The ^{1}H-NMR spectrum showed the signals due to six tertiary methyl groups [δ: 0.79(3H), 0.87(3H), 0.95(6H), 1.3(6H)] along with one secondary methyl group [δ: 0.84, 3H]. The presence of cyclopropane ring was confirmed by the presence of characteristic double doublet at δ 0.54 and 0.60 (J = 3.7 Hz) for two non-equivalent protons at C-19. A multiplet at δ 5.60 integrated for two protons was indicative of vinylic protons at C-23 and C-24, while the carbinylic proton resonated as a pair of doublet at δ 3.27 ($J_{ax,ax}$ = 10.9 Hz, $J_{ax,eq}$ = 4.5 Hz).

On the basis of physical constants and spectral data it was identified as cycloart-23-ene,25,3β -diol (**67**), reported earlier from Tricholepis glaberrime [251].



(67)

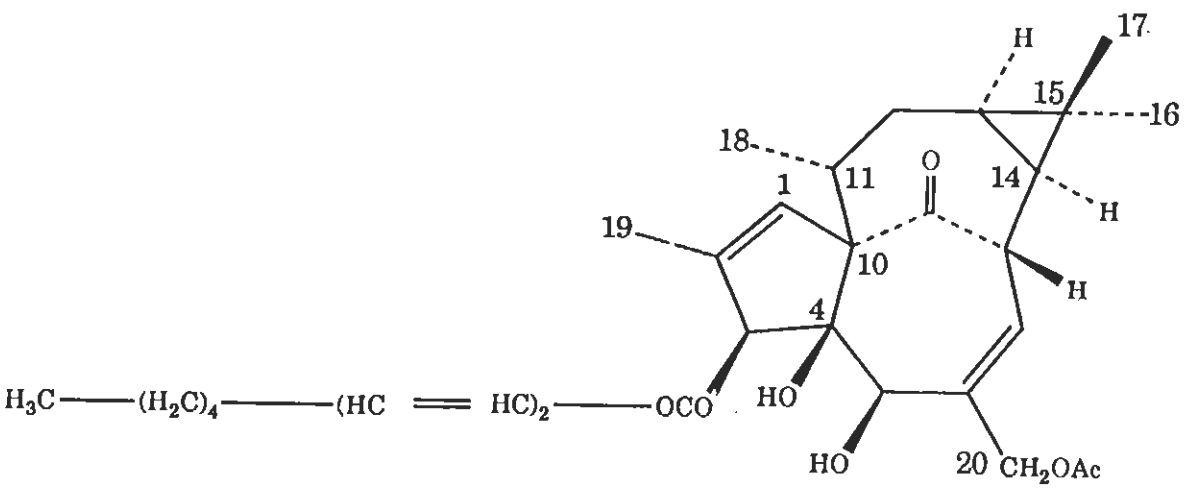
STRUCTURE ELUCIDATION OF 20-ACETYL-INGENOL-3-DECADIENOATE (68)

20-Acetyl-ingenol-3-decadienoate was obtained as an oily product from hexane-chloroform (7:3) eluate and showed $[\alpha]_D$-3.92 ($CHCl_3$). It showed molecular ion peak in high resolution mass spectrum (HRMS) at m/z 540.3063 corresponding to the molecular formula $C_{32}H_{44}O_7$ (calcd. 540.3075) and eleven double bond equivalents in the molecule. The molecular ion peak was also confirmed by field desorption mass spectrometry (FDMS). It gave pink coloration with ceric sulfate and Ehrlich reagent indicating its diterpenic nature. The IR spectrum of the compound showed absorptions at 3680 (OH group), 1730 (-CO- group), 3045 (cyclopropane ring), and 3020 and 1620 (C=C).

The ^{1}H-NMR spectrum of 20-acetyl-ingenol-3-decadienoate showed signals for olefinic protons at δ 7.35 (1H, m, H-4'), 6.65 (1H, dd, J = 10.78 Hz and 10.93 Hz, H-3'), 6.21 (1H, s, H-7), 6.18 (1H, m, H-5'), 6.05 (1H, q, J = 1.96 Hz, H-1), and 5.62 (1H, d, J = 10.78 Hz, H-2'), a methine proton attached to the carbon bearing the ester functionality at δ 5.58 (1H, s, H-3), a methylene group bearing the acetate moiety at δ 4.64 (2H, ABq, J = 13.21 Hz, H_2-20), and an acetate group at δ 2.06 (3H, s, OAc).

The ^{13}C-NMR spectrum showed 32 carbon atoms; the multiplicities of these were determined by using DEPT experiments [245,246], which revealed the presence of six methyl, six methylene, and twelve methine carbon atoms. The number of quaternary carbons were determined by subtracting these from the broad-band spectrum.

The spectral data of (**68**) was in complete agreement with those previously reported for 20-acetyl-ingenol-3-decadienoate [252]. Previously it was isolated from Euphorbia broteri [252].

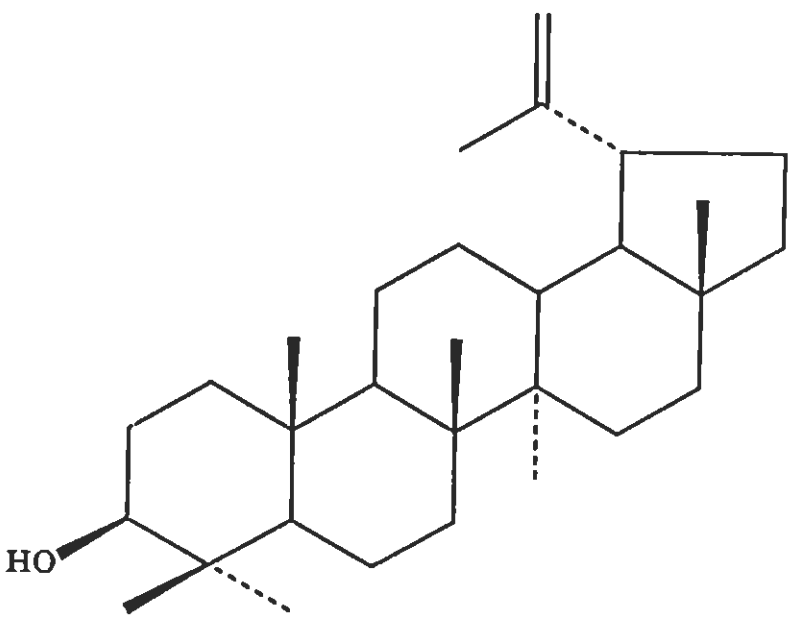


(68)

STRUCTURE ELUCIDATION OF LUPEOL (69)

Lupeol on repeated crystallization from a mixture of acetone methanol formed shining needles, melted at 215 -16°; $[\alpha]_D$ +26.4° ($CHCl_3$). It gave positive Liebermann-Burchard test and violet coloration with ceric sulphate indicating triterpenic nature. It gave the molecular ion peak in its high resolution mass spectrum at m/z 426.6998 corresponding to the molecular formula $C_{30}H_{50}O$ (calcd. 426.7194). The molecular ion peak was also confirmed by field desorption mass spectrum. The IR spectrum showed strong absorption bands for a hydroxyl group at 3440 cm^{-1} and a $C=CH_2$ group at 3070, 1650, 880 cm^{-1}. The ^{1}H-NMR spectrum revealed the presence of seven tertiary methyl groups (singlets at δ 1.59, 1.05, 0.96, 0.90, 0.85, 0.76), along with a multiplet at δ 4.63 which was assigned for olefinic protons. The signal at δ 3.65 (dd, J= 4.27 Hz and J= 10.68 Hz) was attributed to a proton geminal to alcoholic group. Further information about the structure of the compound was provided by its mass spectrum which exhibited typical fragmentation pattern for pentacyclic triterpenes of the lupane series [253].

The ^{13}C-NMR spectrum of the compound showed 30 carbon atoms; the multiplicity assignments of each of these was determined by using DEPT experiments [245,246]. The spectral data of (69) was in complete agreement with those previously reported for lupeol [241]. Previously it has been reported from Euphorbia laterifolia [254].



(69)

STRUCTURE ELUCIDATION OF TARAXASTEROL (70)

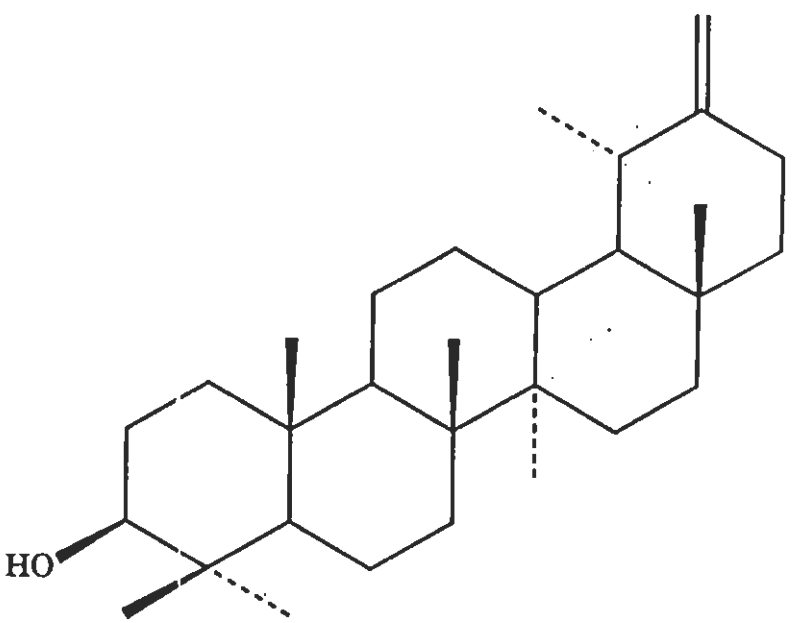
Taraxasterol crystallized from chloroform-methanol as shining needles, melted at 224 - 6° and showed $[\alpha]_D$ +94.80 ($CHCl_3$). It gave positive Liebermann-Burchard test and violet coloration with ceric sulphate indicating triterpenic nature. It gave the molecular ion peak in its high resolution mass spectrum at m/z 426.7165 corresponding to the molecular formula $C_{30}H_{50}O$ (calcd. 426.7194), indicating six double bond equivalents in the molecule.

The IR spectrum showed strong absorption bands for a hydroxyl group at 3420 cm^{-1} a $C=CH_2$ group at 3080, 1650, 880 cm^{-1}.

The ^{1}H-NMR spectrum revealed the presence of six tertiary methyl groups (singlets at δ 0.98, 0.96, 0.94, 0.92, 0.84, and 0.78, 6x CH_3) along with one secondary methyl group (doublet at 1.13, J= 6.57 Hz). The multiplet at δ 4.56 integrated for two protons and was assigned to olefinic protons, while one proton signal at δ 3.18 (dd, J= 4.13 Hz and J= 10.82 Hz) was attributed to the proton geminal to alcoholic group.

Further information about the structure of the compound was provided by the mass spectrum which was characteristic of pentacyclic triterpenes of the taraxasterane series [255].

The ^{13}C-NMR spectrum of the compound showed 30 carbon atoms; the multiplicity assignment of each of these was determined by using DEPT experiments [245,246]. On the basis of comparison of physical constants and spectral data with the literature (242), the compound was identified as taraxasterol (70). Previously it was isolated from Euphorbia watandii (256).



(70)

EXPERIMENTAL

GENERAL NOTES

1.

All melting points were recorded in glass capillary on Gallenkamp melting point apparatus and are uncorrected.

2.

The ultra violet spectra were recorded on Pye Unicam SP-800 spectrophotometer, while infra red spectra were scanned on JASCC A- 302 spectrophotometer.

3.

Electron impact (EI) mass spectra were recorded on Finnigan MAT- 112 and MAT-113 spectrometers coupled with PDP 11/34 computer system. High resolution (HR) mass spectrometry and field desorption (FD) were also performed on MAT-312 mass spectrometer.

4.

^{1}H-NMR spectra were recorded on Bruker AM-300 and AM-400 spectrometers with Aspect 300 data system. The ^{13}C-NMR experiments were performed on the same instruments at 75 and 100-MHz.

5.

Optical rotations were carried out on Schmidt and Haensch polartonic-D polarimeter. Flash column chromatography was performed on Eyela Flash Chromatograph EF-10 model, using silica gel-60 (230- 400 mesh size, E. Merck).

6.

Thin layer chromatography (TLC) was performed on silica gel TLC cards (SIF E. Merck), while column chromatography was performed on silica gel 60 (70-230 mesh, E. Merck).

ISOLATION AND CHARACTERIZATION OF THE CHEMICAL CONSTITUENTS OF EUPHORBIA CLARKEANA

PLANT MATERIAL:

The plant material (20 Kg) was collected from the Karachi District and was identified by Plant Taxonomist, Department of Botany, University of Karachi, where a voucher specimen was deposited.

INVESTIGATION OF EUPHORBIA CLARKEANA

The freshly collected plant material (20 Kg) was chopped into small pieces and then extracted thrice with methanol (60 litres). The combined methanolic extract was evaporated under reduced pressure and the resulting residue was partitioned with water and hexane. The hexane fraction was dried, evaporated, and the resulting residue, which was about 16 g, was then subjected to column chromatography over silica gel (600 g). The elution was successively carried out with increasing polarities of a mixture of hexane and chloroform. The hexane-chloroform eluant (7:3) yielded an oily residue which on further purification by preparative layer chromatography in the same solvent system provided 20-acetyl-ingenol-3-decadienoate (18 mg). The eluates from hexane- chloroform (6:4 and 5.5:4.5) yielded crystalline residues which on repeated crystallization from hexane/chloroform provided cycloartanol (24 mg) and a mixture of triterpenes. The latter could be resolved through flash column chromatography over silica gel using hexane-chloroform (7:3) as solvent system to yield cycloart-23-ene-3β-25-diol (20 mg) and cycloclarkeanol (22 mg), respectively.

The other fraction obtained from hexane- chloroform in different ratios (9:1 to

7.5:2.5) were further separated by preparative tlc using solvent system hexane-chloroform (8.0:2.0) to provide lupeol (12 mg), taraxasterol (16 mg), and α-amyrin (22 mg). These were identified by comparing with data available in literature [241-243].

CHARACTERIZATION OF CYCLOCLARKEANOL (65)

Crystallization from chloroform-methanol provided shining needles of (65); m.p. 190-192°; $[\alpha]_D$ +37.84° (c=0.21, $CHCl_3$).

IR ($CHCl_3$) ν_{max} cm^{-1} : 3460-3440 (OH group), 3065 and 1630 (C=C), 3040 (CH_2 assym. stretching of a cyclopropane ring), 1380 (gem-dimethyl), 1015 (alkyl cyclopropane), and additional bands between 1080-935 (3β-OH and 5α-structure).

EIMS (m/z): (rel.int.%): 458 [$C_{30}H_{50}O_3$, M^+] (18), 443 [$C_{30}H_{50}O_3$- CH_3]$^+$ (16), 440 [$C_{30}H_{50}O_3$-H_2O]$^+$ (38), 425 [$C_{30}H_{50}O_3$-H_2O-CH_3]$^+$ (45), 399 [$C_{30}H_{50}O_3$-$C_2H_3O_2$]$^+$ (16), 318 [$C_{30}H_{50}O_3$-$C_9H_{16}O$]$^+$ (26), 315 [$C_{30}H_{50}O_3$-$C_8H_{15}O_2$]$^+$(32), 175 [$C_{30}H_{50}O_3$-$C_{17}H_{31}O_3$]$^+$ (54).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 5.48 (1H, d, J=7.05 Hz, H-22), 5.40 (1H, m, H-23), 3.26 (1H, dd, $J_{a,a}$= 10.78 Hz and $J_{a,e}$= 4.58 Hz, H- 3), 1.39 (3H, s, Me-21), 1.33 (6H, s, Me-26 and 27), 0.95 (6H, s, Me-18 and 30), 0.87 (3H, s, Me-28), 0.80 (3H, s, Me-29), 0.54- 0.33 (2H, dd, J= 4.20 and 4.26 Hz, H_2-19.

^{13}C-NMR ($CDCl_3$, 100.61 MHz) δ: 31.87 (C-1), 30.26 (C-2), 78.84 (C-3), 40.39 (C-4), 47.03 (C-5), 21.02 (C-6), 28.10 (C-7), 47.89 (C-8), 19.93 (C-9), 26.17 (C-10), 26.01 (C-11), 35.59 (C-12), 45.27 (C-13), 48.82 (C-14), 32.68 (C-15), 26.42 (C-16), 52.10 (C- 17), 17.93 (C-18), 29.81 (C-19), 73.01 (C-20), 24.26 (C-21), 134.47 (C-22), 130.68 (C-23), 39.38 (C-24), 72.28 (C-25), 29.39 (C-26), 29.44 (C-27), 19.22 (C-28), 25.36 (C-29), 14.01 (C-30).

The assignments are made through comparison with published ^{13}C- NMR spectra of related compounds (257,258) and confirmed by ^{1}H- ^{13}C heteronuclear chemical shift correlated spectroscopy (Hetero- COSY).

ACETYLATION OF CYCLOCLARKEANOL. Cycloclarkeanol (5 mg) was dissolved in pyridine (1.0 ml) and refluxed with acetic anhydride (2.5 ml) for 30 minutes. The reaction mixture was worked up in the usual manner. The product (**65a**) was isolated in pure state by preparative tlc over silica gel using hexane- chloroform (6:4) as solvent system.

IR ($CHCl_3$) ν_{max} cm^{-1}: 3460 (OH group), 1715 and 1210 (acetate group),3065 and 1630 (C=C), 3040 (cyclopropane ring), 1380 (gem- dimethyl).

EIMS (m/z): (rel.int.%): 500 [$C_{32}H_{52}O_4$, M^+] (18), 485 [$C_{32}H_{52}O_4$- CH_3]$^+$ (16), 482 [$C_{32}H_{52}O_4$-H_2O]$^+$ (10), 467 [$C_{32}H_{52}O_4$-CH_5O]$^+$ (14), 318 [$C_{32}H_{52}O_4$-$C_{11}H_{18}O_2$]$^+$ (28), 315 [$C_{32}H_{52}O_4$-$C_{10}H_{17}O_3$]$^+$ (24), 175 [$C_{32}H_{52}O_4$-$C_{19}H_{33}O_4$]$^+$ (45).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 5.49 (1H, d, J = 7.10 Hz, H-22), 5.40 (1H, m, H-23), 4.46 (1H, dd, $J_{a,a}$ = 10.75 Hz and $J_{a,e}$ = 4.81 Hz, H- 3), 2.11 (3H, s, OAc), 1.38 (3H, s, Me-21), 1.33 (6H, s, Me-26 and 27), 0.96 (6H, s, Me-18 and 30), 0.89 (3H, s, Me-28), 0.81 (3H s, Me-29), 0.54-0.33 (2H, dd, J =4.21 and 4.24 Hz, H_2-19).

CHARACTERIZATION OF CYCLOARTANOL (66)

Crystallization from 90% methanol provided shining needles of (**66**); m.p. 100-102°; $[\alpha]_D$ +49.7° (c = 0.14, $CHCl_3$).

IR ($CHCl_3$) ν_{max} cm^{-1}: 3430 (OH group), 3045 (cyclopropane ring).

EIMS (m/z): (rel. int.%): 428 [$C_{30}H_{52}O$, M^+] (12), 413 [$C_{30}H_{52}O$-CH_3]$^+$ (18), 410 [$C_{30}H_{52}O$-H_2O]$^+$ (25), 395 [$C_{30}H_{52}O$-CH_3-H_2O]$^+$ (15), 355 [($C_{30}H_{52}O$-H_2O)-69]$^+$ (10), 315 [$C_{30}H_{52}O$-C_8H_{17} (entire substituent at C-17)]$^+$ (25), 288 [$C_{30}H_{52}O$-$C_9H_{16}O$ (loss of ring A along with C-6 or C-19)]$^+$ (30), 273 [$C_{30}H_{52}O$-$C_9H_{16}O$-CH_3]$^+$ (40), 297 [$C_{30}H_{52}O$-C_8H_{17}-H_2O]$^+$ (35) and 175 [$C_{30}H_{52}O$-$C_9H_{19}O$-C_8H_{17}]$^+$ (45).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 0.33-0.56 (2H, dd, J = 4.0 Hz, 19-H), 0.82 (3H, s, Me-29) 0.88 (3H, s, Me-28), 0.96 (6H, s, Me-30 and Me-18), 0.90 (6H, d, J = 6.7 Hz, Me-26 and Me-27), 0.87 (3H, d, J = 7.1 Hz, Me-21) and 3.27 (1H, dd, $J_{ax,ax}$ = 10.50 Hz, $J_{ax,eq}$ = 4.50 Hz, 3-H).

^{13}C-NMR ($CDCl_3$ 100.61 MHz) δ: 31.92 (C-1), 30.34 (C-2), 78.62

(C-3), 40.31 (C-4), 47.09 (C-5), 21.01 (C-6), 28.10 (C-7), 47.87 (C-8), 20.10 (C-9), 26.21 (C-10), 26.00 (C-11), 35.62 (C-12), 45.12 (C-13), 48.79 (C-14), 32.71 (C-15), 26.50 (C-16), 52.21 (C-17), 17.91 (C-18), 29.81 (C-19), 36.00 (C-20), 18.30 (C-21), 36.41 (C-22), 24.01 (C-23), 39.40 (C-24), 28.20 (C-25), 22.51 (C- 26), 22.71 (C-27), 19.30 (C-28), 25.41 (C-29), and 14.10 (C-30).

CHARACTERIZATION OF CYCLOART-23-ENE-3β,25-DIOL (67)

Crystallization from acetone provided shining needles of (67); m.p. 196-98°; $[\alpha]^{20}_{D}$ + 33.90° (c = 0.2, $CHCl_3$).

IR ($CHCl_3$) ν_{max} cm^{-1}: 3590, 3430 (OH group), 3040 (cyclopropane ring).

EIMS (m/z): (rel. int. %): 442 [$C_{30}H_{50}O_2$,M^+] (12), 427 [$C_{30}H_{50}O_2$- CH_3]$^+$ (10), 424 [$C_{30}H_{50}O_2$-H_2O]$^+$ (15), 409 [$C_{30}H_{50}O_2$-H_2O-CH_3]$^+$ (20), 391 [$C_{30}H_{50}O_2$-2H_2O-CH_3]$^+$ (18), 302 [$C_{30}H_{50}O_2$ - $C_9H_{16}O$ (loss of ring A along with C-6 or C-19)]$^+$ (40), 315 [$C_{30}H_{50}O_2$-$C_8H_{18}O$ (entire substituent at C-17)]$^+$ (35), 294 [$C_{30}H_{50}O_2$-$C_8H_{18}O$-H_2O]$^+$ (40), 287 [$C_{30}H_{50}O_2$-$C_{10}H_{19}O$]$^+$ (62), and 175 [$C_{30}H_{50}O_2$-$C_8H_{18}O$- $C_9H_{16}O$]$^+$ (75).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 0.54-0.60 (2H, dd, J = 3.7 Hz, 19-H), 0.79 (3H, s, Me-29), 0.84 (3H, d, J = 6.9 Hz, Me-21), 0.87 (3H, s, Me-14), 0.95 (6H, s, Me-30 and Me-13), 1.3 (6H, s, Me-26 and Me-27), 3.27 (1H, dd, $J_{ax,ax}$ = 10.9 Hz, $J_{ax,eq}$ = 4.5 Hz, 3-H), 5.60 (2H, m, 23-H and 24-H).

^{13}C-NMR ($CDCl_3$, 100.61 MHz) δ: 31.85 (C-1), 30.27 (C-2), 78.74 (C-3), 40.37 (C-4), 46.98 (C-5), 20.98 (C-6), 27.96 (C-7), 47.87 (C-8), 19.87 (C-9), 25.97 (C-10), 25.89 (C-11), 35.46 (C-12), 45.20 (C-13), 48.72 (C-14), 32.67 (C-15), 26.32 (C-16), 51.90 (C-17), 17.97 (C-18), 29.78 (C-19), 36.27 (C-20), 18.16 (C-21), 38.90 (C-22), 125.50 (C-23), 139.20 (C-24), 70.64 (C-25), 29.76 (C-26), 29.85 (C-27), 19.17 (C-28), 25.30 (C-29), 13.86 (C-30).

CHARACTERIZATION OF 20-ACETYL-INGENOL-3-DECADIENOATE (68)

The hexane- chloroform (7:3) eluate obtained from silica gel column yielded (**65**) (18 mg) as an oily product; $[\alpha]_D$ -3.92° (c = 0.21, $CHCl_3$).

IR ($CHCl_3$) ν_{max} cm^{-1}: 3680 (OH group), 1730 (-CO- group), and 3040, 1620 (C = C).

EIMS (m/z): (rel. int. %): 540 [$C_{32}H_{44}O_7$, M^+] (5), 522 [$C_{32}H_{44}O_7$- H_2O]$^+$ (3), 480 [$C_{32}H_{44}O_7$-$C_2H_4O_2$]$^+$ (4), 390 [$C_{32}H_{44}O_7$-$C_{10}H_{14}O$]$^+$ (25), 374 [$C_{32}H_{44}O_7$-$C_{10}H_{14}O_2$]$^+$ (5), 330 [$C_{32}H_{44}O_7$-$C_{11}H_{14}O_4$]$^+$ (20).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.35 (1H, m, H-4'), 6.65 (1H, dd, J = 10.78 Hz and 10.93 Hz, H-3'), 6.21 (1H, s, H-7), 6.18 (1H, m, H- 5'), 6.05 (1H, q, J = 1.96 Hz, H-1), 5.62 (1H, d, J = 10.78 Hz, H- 2'), 5.58 (1H, s, H-3), 4.64 (2H, ABq, J = 13.21 Hz, H_2-20), 4.12 (1H, dd, J = 4.58 Hz and 11.78 Hz, H-8), 3.85 (1H, s, H-5), 2.06 (3H, s, OAc), 1.83 (3H, d, J = 1.78 Hz, Me-19), 1.07 (3H, s, Me- 17), 1.04 (3H, s, Me-16), 0.99 (3H, d, J = 7.21 Hz, Me-18), 0.89 (3H, t, Me-10')

^{13}C-NMR ($CDCl_3$, 100.61 MHz) δ: 132.21 (C-1), 136.10 (C-2), 82.53 (C-3), 85.06 (C-4), 75.01 (C-5), 136.17 (C-6), 129.36 (C-7), 43.76 (C-8), 206.12 (C-9), 72.23 (C-10), 38.55 (C-11), 31.32 (C- 12), 23.45 (C-13), 23.26 (C-14), 24.01 (C-15), 28.53 (C-16), 15.49 (C-17), 17.32 (C-18), 15.53 (C-19), 66.76 (C-20), 166.83 (C-1'), 114.39 (C-2'), 147.16 (C-3'), 127.01 (C-4'), 147.12 (C-5'), 33.06 (C-6'), 28.51 (C-7'), 31.47 (C-8'), 22.46 (C-9'), 13.96 (C-10'), 170.86 (CO-CH_3), 21.17 (CO-CH_3).

CHARACTERIZATION OF LUPEOL (69)

Colorless needles from acetone-methanol; yield 13 mg; m.p. 215- 16°; $[\alpha]_D + 26.40^\circ$ (c = 0.21, $CHCl_3$).

IR (KBr) ν_{max} cm^{-1}: 3350 (OH group), 3070, 1650, and 880 (double bond).

EIMS (m/z): (rel.int., %) : 426 $[C_{30}H_{50}O, M^+]$ (14), 411 $[C_{30}H_{50}O - CH_3]^+$ (30), 207 $[C_{30}H_{50}O - C_{16}H_{29}]^+$ (10), 206 $[C_{30}H_{50}O - C_{16}H_{28}]^+$ (1), 205 $[C_{30}H_{50}O - C_{16}H_{29}]^+$ (1), 204 $[C_{30}H_{50}O - C_{16}H_{30}]^+$ (7), 203 $[C_{30}H_{50}O - C_{16}H_{31}]^+$ (6), 189 $[C_{30}H_{50}O - C_{16}H_{29}O]^+$ (20), 133 $[C_{30}H_{50}O - C_{20}H_{37}O]^+$ (11).

^{1}H-NMR ($CDCl_3$, 300 MHz) δ : 4.63 (2H,m,29-H_2), 3.65 (1H,dd,J = 4.28 and 10.72 Hz, 3-H), 1.56 (3H,s,30-CH_3), 1.03 (3H,s,26-CH_3), 0.96 (6H,s,25,27-CH_3), 0.90 (3H,s,24-CH_3), 0.85 (3H,s,28-CH_3), 0.76 (3H,s,23-CH_3).

^{13}C-NMR ($CDCl_3$, 75 MHz) δ : 38.71 (C-1), 27.43 (C-2), 78.81 (C- 3), 38.84 (C-4), 55.27 (C-5), 18.36 (C-6), 34.25 (C-7), 40,96 (C- 8), 50.49 (C-9), 37.19 (C-10), 20.91 (C-11), 25.12 (C-12), 38.03 (C-13), 42.81 (C-14), 27.44 (C-15), 35.51 (C-16), 42.91 (C-17), 48.21 (C-18), 47.91 (C-19), 150.61 (C-20), 29.84 (C-21), 39.94 (C-22), 28.04 (C-23), 15.41 (C-24), 16.11 (C-25), 15.93 (C-26), 14.51 (C-27), 18.09 (C-28), 109.21 (C-29), 19.30 (C-30).

CHARACTERIZATION OF TARAXASTEROL (70)

Repeated crystallization from chloroform-methanol resulted in colorless needles of taraxasterol (11 mg); m.p. 224-26°, $[\alpha]_D$ +94.80° (c = 0.18, $CHCl_3$).

IR (KBr) ν_{max} cm^{-1}: 3420 (OH group), 3080, 1650, and 880 (double bond).

EIMS (m/z): (rel.int., %) : 426 [$C_{30}H_{50}O$,M^+] (72), 411 [$C_{30}H_{50}O$ - CH_3]$^+$ (7), 408 [$C_{30}H_{50}O$ - H_2O]$^+$ (16), 393 [$C_{30}H_{50}O$ - H_2O-CH_3]$^+$ (12), 357 [$C_{30}H_{50}O$ - C_5H_9]$^+$ (15), 315 [$C_{30}H_{50}O$ - C_8H_{15}]$^+$ (10), 207 [$C_{30}H_{50}O$ -$C_{16}H_{27}$]$^+$ (40), 189 [$C_{30}H_{50}O$ - $C_{16}H_{29}O$]$^+$ (80), 135 [$C_{30}H_{50}O$ - $C_{20}H_{35}O$]$^+$ (45).

^{1}H-NMR ($CDCl_3$, 300 MHz) δ : 4.63 (2H,m,29-H_2), 3.65 (1H,dd,J = 4.27 Hz and J = 10.68 Hz, 3-H), 1.59 (3H,s,30-CH_3), 1.05 (3H,s, 26-CH_3), 0.96 (6H,s,25,27-CH_3), 0.90 (3H,s,24-CH_3), 0.85 (3H,s,28-CH_3), 0.76 (3H,s,23-CH_3).

^{13}C-NMR ($CDCl_3$, 75 MHz) δ : 38.71 (C-1), 27.43 (C-2), 78.81 (C- 3), 38.84 (C-4), 55.27 (C-5), 18.36 (C-6), 34.25 (C-7), 40.86 (C- 8), 50.49 (C-9), 37.19 (C-10), 20.91 (C-11), 25.12 (C-12), 38.03 (C-13), 42.81 (C-14), 27.44 (C-15), 35.51 (C-16), 42.91 (C-17), 48.21 (C-18), 47.91 (C-19), 150.61 (C-20), 29.84 (C-21), 39.94 (C-22), 28.04 (C-23), 15.41 (C-24), 16.11 (C-25), 15.93 (C-26), 14.51 (C-27), 18.09 (C-28), 109.21 (C-29), 19.30 (C-30).

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PART - B

RESULTS AND DISCUSSIONS

Sericostoma is a small genus of family Boraginaceae. It comprises of eight species distributed through the tropical east and north- east of Africa to north-west India. These have close morphological resemblance to Heliotropium species which are reputed to possess antitumor, carcinogenic, diuretic, laxative, and emetic activities [1,2]. In Pakistan this genus is represented by only one species namely Sericostoma pauciflorum which is a short straggling under shrub [3]. A carefurl search of literature survey revealed that no report of chemical work on species of the genus Sericostoma has appeared. Therefore, in view of the possible medicinal potentials of Sericostoma species, systematic chemical studies have been carried out by us on Sericostoma pauciflorum.

The present work has been undertaken on the freshly collected plant material from the Karachi District with particular reference to the isolation structural elucidation of new terpenoids. The techniques employed for the purpose follow the traditional procedures as well as various forms of adsorption chromatography.

The freshly collected plant material was chopped into small pieces and then extracted thrice with methanol. The combined methanolic extract was evaporated under reduced pressure to afford a gummy residue. This residue was partitioned between hexane and water. The residue recovered from hexane fraction was chromatographed over activated silica gel column and the elution was carried out with solvent gradients of increasing polarities of a mixture of hexane and chloroform. The eluates obtained from hexane-chloroform in the ratio of 9.5:0.5 and 9:1 yielded β-amyrin acetate (**6**) and β-sitosterol (**7**), respectively. The eluate from hexane-chloroform (8:2) yielded a gummy residue which was found to be a mixture of

terpenes by thin layer chromatography. These were resolved through flash column chromatography over silica gel using hexane-chloroform (9:1) as solvent system to yield α- and β- amyrins (**4,5**), pauciflorinyl acetate (**2**), and pauciflorol acetate (**3**). On the other hand, the eluate obtained from hexane-chloroform in the ratio of (7:3) yielded sericostinyl acetate (**1**).

STRUCTURE ELUCIDATION OF SERICOSTINYL ACETATE

[ferna-7,9(11)-dien-20β-yl-acetate] (**1**)

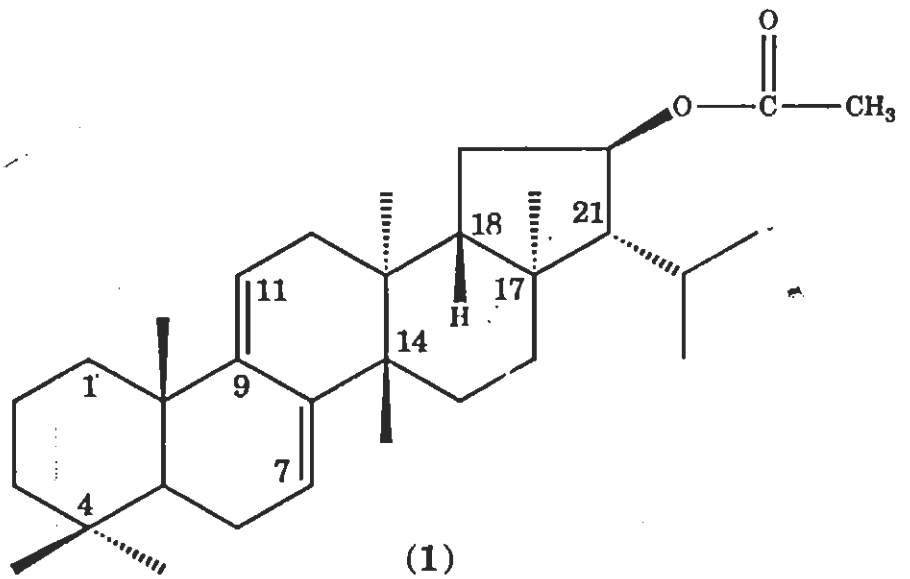
Sericostinyl acetate formed colorless shining needles on repeated crystallization from hexane / chloroform, melted at 268-70^{o} and showed $[\alpha]_D$-12.34^{o} ($CHCl_3$). It showed molecular ion peak in high resolution mass spectrum (HRMS) at m/z 466.3799 corresponding to the molecular formula $C_{32}H_{50}O_2$ (calcd. 466.3798) and eight double bond equivalents in the molecule. The molecular ion peak was also confirmed by field desorption mass spectrometry (FDMS). Sericostinyl acetate gave positive Liebermann-Burchard test and violet coloration with ceric sulfate indicating its triterpenic nature. It also gave a tetranitromethane color reaction for unsaturation. The IR spectrum of the compound showed absorptions at 1710, 1220 (ester group), 1660 (conjugated trisubstituted C=C), and 1325, 1310 cm^{-1} (gem-dimethyl group). The UV maxima at 232, 237, 250, and 258 nm were characteristic for 7,9 (11)-dienes with 13α, 14β, methyl groups [9].

The ^{1}H-NMR spectrum of sericostinyl acetate showed signals for two trisubstituted double bonds (dds at δ 6.17 and 5.45), an acetate group (s at δ 2.04), a proton geminal to the acetoxy group (q at δ 4.49), two secondary methyl groups (doublets at δ 0.85 and 0.94), and six tertiary methyl groups.

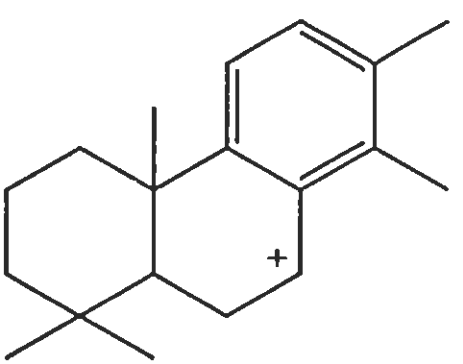
The ^{13}C-NMR spectrum indicated the presence of 32 carbon atoms; their multiplicities were determined by DEPT experiments keeping the last pulse angle $\theta = 45^{o}, 90^{o}$ and 135^{o} [10,11]. Sericostinyl acetate showed the presence of 9-methyl, 8-methylene and 7- methine carbons. The number of quaternary carbons were determined by subtracting these from the broad band spectrum.

The high resolution mass spectrum showed diagnostic fragments at m/z 451.3501 ($C_{32}H_{50}O_2$-CH_3), 406.3625 ($C_{32}H_{50}O_2$-$C_2H_4O_2$) and 423.3291 ($C_{32}H_{50}O_2$-C_3H_7), resulting from loss of a methyl group, an acetic acid and an isopropyl moiety, respectively. Another ion at m/z 391.3353 represented simultaneous loss of a methyl group and acetic acid. The base peak at m/z 255.2115 ($C_{19}H_{27}$) was due to ion "a" characteristic of ferna-7,9(11)-diene or arboradiene carrying acetoxy group in rings D/E [9]. The presence of isopropyl group, however, allowed us to assign fernane type skeleton to (**1**), the only problem remaining being the allocation of acetoxy group. A careful comparison of the ^{1}H-NMR spectrum of **1** with that of ferna-7,9(11)-diene [12] showed that of the eight methyl signals in the two compounds five at 4,4,10,13 and 14 have good agreement revealing similarity in structure and stereochemistry of rings A-D. The downfield shift of three other methyl signals at 17,22,22 in case of sericostinyl acetate should be due to the influence of the acetoxy group in ring E. Such downfield shifts of these methyl groups have already been reported for fern-9(11)- en-20-β-ol [13].

The presence of an acetoxy function in ring E at C-20 in β- and equatorial orientation was finally demonstrated by a combination of selective homodecoupling experiments, ^{1}H-^{1}H- and ^{1}H-^{13}C- correlated spectroscopy. The proton geminal to acetoxy group at δ 4.49 showed cross peaks with proton signals at δ 1.65 and δ 1.17 in cosy-45^o spectrum. The latter in turn showed cross peaks with carbon signals at δ 59.30 and 23.56 in heterocosy spectrum which were found to be methylene and methine carbons by DEPT experiments, thereby confirming the presence of acetoxy group at position 19/20. Irradiation at either of the methyl doublets simplified the multiplet at δ 1.19 which could, therefore, be assigned to methine proton of the isopropyl moiety.



(1)



(a)

On the other hand, irradiation at δ 1.19 caused the double doublet at δ 1.17 to collapse into a doublet showing an axial-axial coupling of 6.21 Hz with the neighbouring proton. The signal at δ 1.17 could, therefore, be assigned for β- and axial proton at C-21. The connectivity of this proton to the proton geminal to the acetoxy group confirmed the position of the latter at C-20 in β- and equatorial orientation. The stereostructure of sericostinyl acetate could, therefore, be represented by (**1**).

The fernane type triterpenes are a rare class of compounds which, to date, have been isolated only from ferns. The present report constitutes the first example of their occurrence in other plants which may be of chemotaxonomic importance.

STRUCTURE ELUCIDATION OF PAUCIFLORINYL ACETATE

[hopan-24-ol-20β-yl-acetate] (2)

Pauciflorinyl acetate formed colorless shining needles on crystallization from a mixture of hexane/chloroform, melted at 240- 42° and showed $[\alpha]_D + 47.6^\circ$ ($CHCl_3$). It showed molecular ion peak in high resolution mass spectrum (HRMS) at m/z 486.3984 corresponding to the molecular formula $C_{32}H_{54}O_3$ (calcd. 486.4059) and six double bond equivalents in the molecule. The molecular ion peak was also confirmed by field desorption mass spectrometry (FDMS). Pauciflorinyl acetate gave positive Liebermann-Burchard test and violet coloration with ceric sulfate indicating its triterpenic nature. The IR spectrum of pauciflorinyl acetate showed absorptions at 3400-3350 (OH group), 1715, 1210 (ester group), 1325 cm^{-1} (gem-dimethyl group).

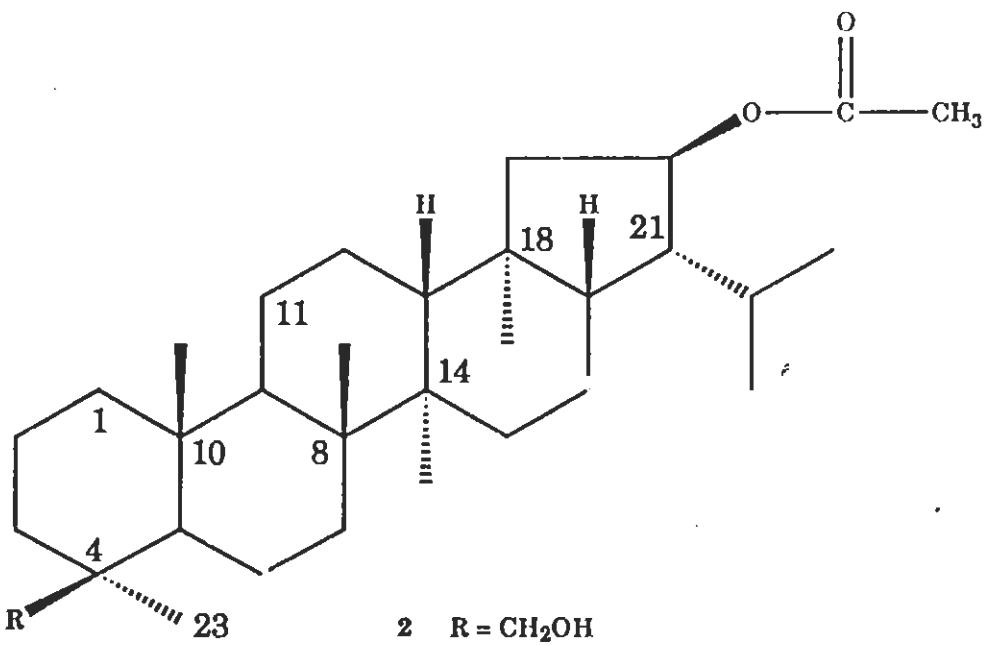
The ^{1}H-NMR spectrum of pauciflorinyl acetate showed signals due to an acetate group (s at δ 2.03), a proton geminal to the acetoxy group (m at δ 4.46), five tertiary methyl groups (singlets at δ 0.81,0.82,0.97,0.99 and 1.02), and two secondary methyl groups (d at δ 0.84 and 0.95). In addition a methylene group at δ 3.62 (ABq, J = 13.12 Hz) was attributed to a primary alcohol with no proton on the adjacent carbon atom.

The ^{13}C-NMR spectrum of pauciflorinyl acetate showed 32 carbon atoms. The multiplicity assignment of each carbon atom was determined by using DEPT experiments [10,11] which revealed the presence of 8-methyl, 11-methylene, and 7-methine carbons. The number of quaternary carbons were determined by subtracting these spectra from the broad band ^{13}C-NMR spectrum.

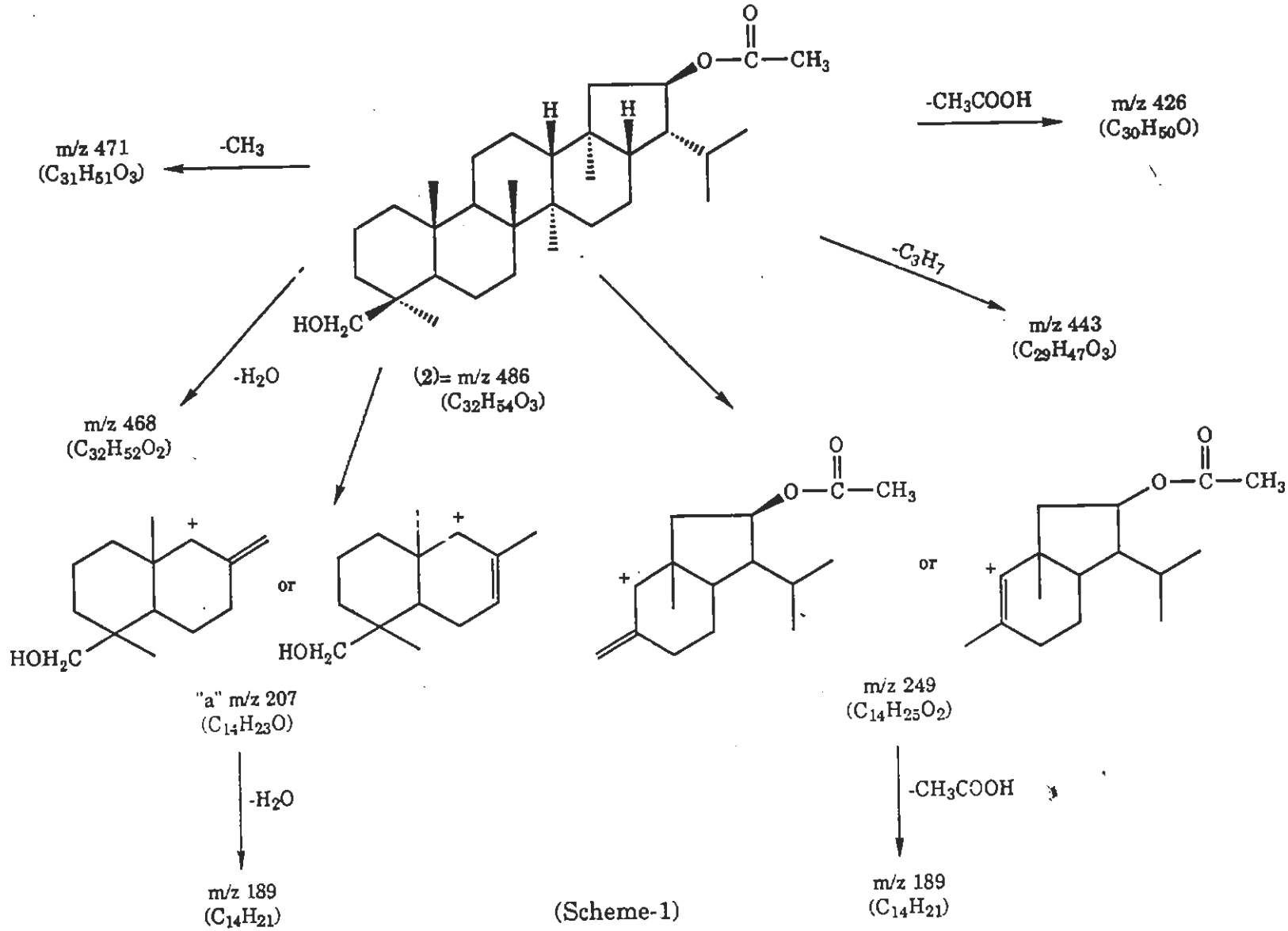
A wealth of structural information of pauciflorinyl acetate (2) was provided by the

mass spectrum of **2** which was characteristic of pentacyclic triterpenes of the saturated hopane series. The genesis of the various fragments was confirmed by link scan measurements. The peaks at m/z 471.3781, 468.3879, 426.3851 and 443.3538 originated from the molecular ion peak by the loss of the methyl group, water, acetic acid, and isopropyl moiety, respectively. The characteristic ion at m/z 207.1735 ($C_{14}H_{23}O$) was generated by cleavage "a". Further ion at m/z 189.1630 ($C_{14}H_{21}$) originated from the ion at m/z 207.1735 by the loss of water molecule. Both these fragments have similar composition as those observed for the corresponding ions of hopan-3β-ol [14] showing that pauciflorinyl acetate is an isomer of hopan-3β-ol with respect to rings A and B, the former differing from the latter in having a primary alcoholic function instead of secondary. Another characteristic ion at m/z 249.1890 ($C_{16}H_{25}O_2$) could be attributed to cleavage "b". The same ion in hopane and hopan-3β-OI [14] occurs at m/z 191 revealing the presence of acetate moiety in ring D/E. This was further confirmed by the loss of acetic acid from this ion to give a strong peak at m/z 189.1630 ($C_{14}H_{21}$) which also originated through cleavage "a".

The absence of species at M-31 for pauciflorinyl acetate indicated that the primary hydroxyl group is not at an angular position. It must, therefore, be assigned to C-23 or C-24. The latter was proved to be correct by ^{1}H NMR data for the methylene group in **2** which agreed with that for axial -CH_2OH [15]. Further evidence to this effect was provided by oxidation product (**2a**). Its ^{1}H NMR spectrum showed the signal of the aldehydic proton at δ 9.75 corresponding to the axial orientation [16]. The position and stereochemistry of the hydroxyl group was finally confirmed by the long range ^{1}H-^{13}C correlated spectrum (COLOC) of **2a** which showed a cross peak between aldehydic carbon and the methyl protons of C-23 confirming the presence of



2 R = CH_2OH
2a R = CHO
2b R = COOH


m/z 471
(C31H51O3)
-CH3
-CH3COOH
m/z 426
(C30H50O)
-C3H7
m/z 443
(C29H47O3)
-H2O
m/z 468
(C32H52O2)
(2)= m/z 486
(C32H54O3)
or
"a" m/z 207
(C14H23O)
-H2O
m/z 189
(C14H21)
or
m/z 249
(C14H25O2)
-CH3COOH
m/z 189
(C14H21)

(Scheme-1)

aldehydic group in **2a** and hence hydroxymethyl group in **2** in β-and axial orientation.

The remaining problem is the allocation of the acetoxy group in ring D/E. A careful comparison of the ^{1}H- and ^{13}C NMR spectra of **2** with those of hopane and hopan-3β-Ol showed that of the seven methyl signals in these compounds four at 8,10,14 and 18 have good agreement revealing similarity in stereochemistry of rings A-D. The methyl group at 23 showed an upfield shift of about 4.5 ppm comparing to hopane [14,17] which could be attributed to the shielding effect of the primary alcoholic group. The upfield shift of such a magnitude is in close agreement to that reported earlier in literature for urs-12(13)-en-24β-ol which is a hydroxyl isomer of α-amyrin [18]. The variation in chemical shifts of two other methyl signals at 22,22 in the case of **2** should be due to the influence of the acetoxy group in ring E.

The presence of an acetoxy function in ring E at C-20 in β- and equatorial orientation was finally demonstrated by a combination of selective homodecoupling experiments using ^{1}H-^{1}H- and ^{1}H-^{13}C correlated spectroscopy. The proton geminal to the acetoxy group at δ 4.46 showed cross peaks with proton signals at δ 1.54 and δ 1.17 in the COSY-45^{o} spectrum. The latter in turn showed cross peaks with carbon signals at δ 43.28 and 48.75 in the hetero-COSY spectrum, these were found to be methylene and methine carbons by DEPT experiments, thereby confirming the presence of an acetoxy group at position 16 or 20. Irradiation at either of the mehyl doublets simplified the multiplet at δ 1.21 which could, therefore, be assigned to the methine proton of the isopropyl moiety. On the other hand, irradiation at δ 1.21 caused the multiplet at δ 1.17 to collapse into a double doublet showing axial-axial coupling of 7.12 Hz and axial- equatorial coupling of 3.32 Hz with the neighbouring protons. The

signal at δ 1.17 could, therefore, be assigned for β- and the axial proton at C-21. The connectivity of this proton to the β- and equatorial proton at C-17 and the proton geminal to the acetoxy group confirmed the position of the acetoxy group at C-20 in β-and equatorial orientation. The stereostructure of paucifiorinyl acetate and its oxidation products could, therefore, be represented by **2**.

STRUCTURE ELUCIDATION OF PAUCIFLOROL ACETATE

[3β,25-epoxy-hopan-20β-yl-acetate] (3)

Pauciflorol acetate formed colorless shining needles on repeated crystallization from ether/ethyl acetate melted at 264-66^{o} and showed $[\alpha]_D + 58.20^{o}$ ($CHCl_3$). It showed molecular ion peak in high resolution mass spectrum (HRMS)at m/z 484.3918 corresponding to the molecular formula $C_{32}H_{52}O_3$ (calcd. 484.3903) and seven double bond equivalents in the molecule. The molecular ion peak was also confirmed by field desorption mass spectrometry (FDMS). Pauciflorol acetate was saturated to tetranitromethane, showed no high intensity absorption in the UV, gave positive Liebermann Burchard test and a violet coloration with ceric sulfate, suggesting the triterpenic nature. The IR spectrum showed peaks at 1710, 1220 (ester), 1330, 1310 (gem.dimethyl), 1089 and 813 cm^{-1} (ether).

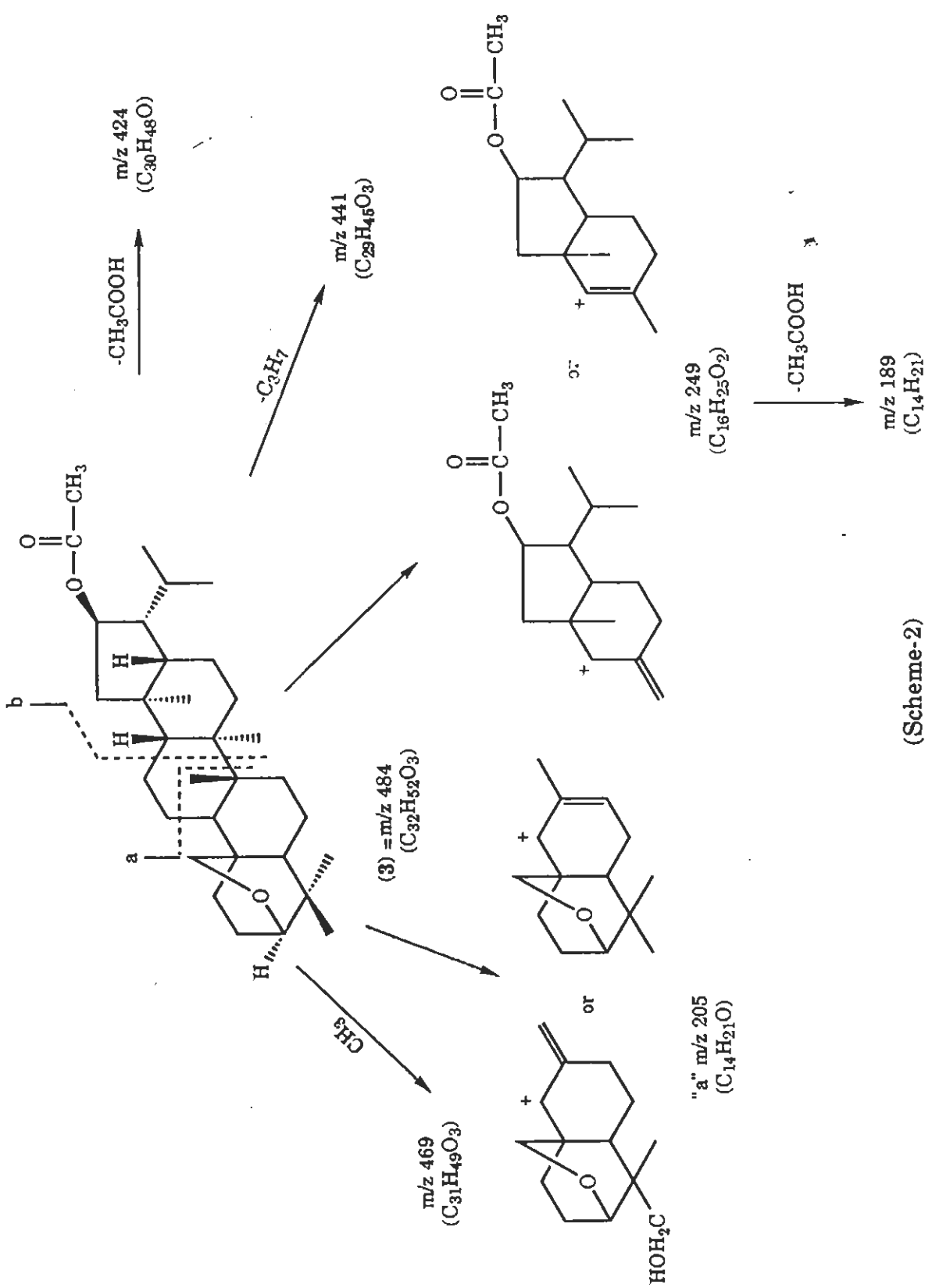
The ^{1}H-NMR spectrum showed signals due to an acetate group (3H,s, at δ 2.15), a proton geminal to the acetoxy group (multiplet at δ 4.48), a proton geminal to an oxygen function (double doublet at δ 3.79, J= 9.72 and 3.95 Hz), five tertiary methyl groups (singlets at δ 0.80,0.81,0.83,0.94 and 0.97) and two secondary methyl groups (doublets at δ 0.84 and 0.95, respectively). It also showed that the ether function is of the type CH_2-O-C(AB quartet with doublets centred at δ 3.59 and 3.79, J_{gem}= 12.17 Hz).

The ^{13}C-NMR spectrum showed the presence of 32 carbon atoms; their multiplicity was determined by using DEPT experiments [10,11] with the last polarization pulse angle θ = 45^{o}, 90^{o}, and 135^{o}. It revealed the presence of 8 methyl, 10 methylene and 8 methine carbons. The number of quaternary carbons were determined by

subtracting these from the broad-band decoupled spectrum.

A wealth of structure information was provided by mass fragmentation pattern of (**3**) which was characteristic of pentacyclic triterpenes of the saturated hopane series [14]. The genesis of various fragments was confirmed by link-scan measurements. The peaks at m/z 469.3680, 424.3723, and 441.3445 originated from the molecular ion peak by the loss of methyl group, acetic acid, and an isopropyl moiety, respectively. Another Ion at m/z 409.3504 represented the simultaneous loss of a methyl group and acetic acid. The ion at m/z 205.1598 ($C_{14}H_{21}O$) was generated by cleavage "a". The same ion in hopan-3β-ol occurs at m/z 207 [14] and represents rings A and B. In view of absence of unsaturation cyclic ether was inferred in pauciflorol acetate in ring A/B. Another ion at m/z 249.1886 ($C_{16}H_{25}O_2$) could be attributed to cleavage "b". The same peak occurs in hopane and hopan-3-β-ol at m/z 191 [14] revealing the presence of acetate moiety in ring D/E. This was further confirmed by the loss of acetic acid form this ion to give a strong peak at m/z 189.1632 ($C_{14}H_{21}$).

A careful comparison of the ^{1}H- and ^{13}C-NMR spectra of (**3**) with those of hopane and hopan-3β-ol [14,17], particularly with respect to signals of rings C and D, showed that of the four methyl signals in these compounds two at 14 and 18 have good agreement revealing similarity in stereochemistry at these centres. The variation of chemical shifts of two other methyl signals at 22,22 in the case of (**3**) should be due to the influence of the acetoxy group in ring E. Such downfield shifts of these methyl groups have already been reported for fern-9(11)-en-20-β-ol [13]. The position of acetoxy group at C-20 in β- and equatorial orientation was finally demonstrated by a


-CH3COOH
m/z 424
(C30H48O)
-C3H7
m/z 441
(C29H45O3)
(3) =m/z 484
(C32H52O3)
CH3
m/z 469
(C31H49O3)
or
"a" m/z 205
(C14H21O)
HOH2C
m/z 249
(C16H25O2)
-CH3COOH
m/z 189
(C14H21)

(Scheme-2)

combination of selective homodecoupling experiments using ^{1}H-^{1}H- and ^{1}H-^{13}C-correlated spectroscopy. The proton geminal to the acetoxy group at δ 4.48 showed cross peaks with proton signals at δ 1.52 and δ 1.18 in the COSY-45^{O} spectrum. The latter in turn showed cross peaks with carbon signals at δ 43.29 and 48.76 in the hetero-COSY spectrum, these were found to be methylene and methine carbons by DEPT experiments, thereby confirming the presence of an acetoxy group at position 16 or 20. Irradiation at either of the methyl doublets simplified the multiplet at δ 1.21 which could, therefore, be assigned to the methine proton of the isopropyl moiety. On the other hand, irradiation at δ 1.21 caused the multiplet at δ 1.18 to collapse into a double doublet showing axial-axial coupling of 7.21 Hz and axial equatorial coupling of 3.31 Hz with the neighbouring protons. The signal at δ 1.18 could thus be assigned to β- and axial proton at C-21. The connectivity of this proton to the β- and equatorial proton at C-17 and the proton geminal to the acetoxy group confirmed the position of the acetocy group at C-20 in β- and equatorial orientation.

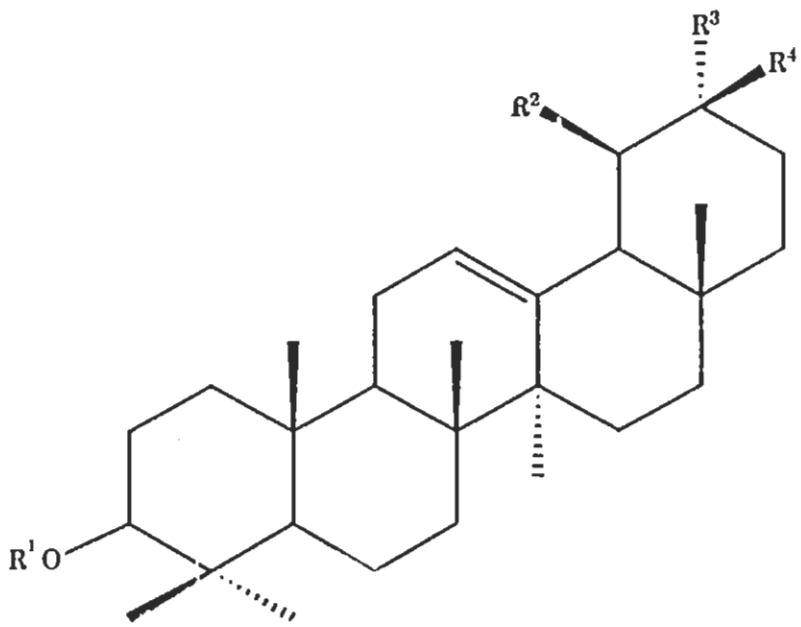
The remaining problem is location of points of attachment of ethereal exygen. Comparison of the ^{1}H- and ^{13}C-NMR spectra of (3) with those of hopane and hopan-3β-ol [14,17], with respect to signals of rings A and B, showed that three tertiary methyl signals at 4,4 and 8 have close agreement while the signal of C- 10 methyl was altogether absent. The two proton AB quartet with double doublets at δ 3.59 and 3.79 could, therefore, be assigned to non-equivalent protons at C-25. The other proton geminal to oxygen at δ 3.79 showed cross peaks with two other protons in COSY-45^{O} spectrum which were found to be joined to the same carbon atom by hetero-COSY and DEPT experiments. It must therefore be at positions 1,3, or 7 in axial orientation as shown by values of its couplings with those protons (9.72 and

3.95 Hz). The position 7 was excluded by the fact that it is involved in facile formation of ion "a" which would be highly unlikely if ethereal oxygen is attached to it. The attachment to position 1 would give a 4-membered cyclic ether of lesser stability while position-3 is highly favourable not only biogenetically but also by stability considerations. Moreover, C-O symmetrical and assymetrical stretching frequencies in the IR spectrum corresponded to a 6-membered cyclic ether rather than 4-membered [20]. This was further authenticated by chemical shifts in ^{1}H- and ^{13}C-NMR spectra of C-4 and the two methyls attached to it. If point of attachment would have been other than position-3 then these three signals must have occurred upfield as in the case of hopane [14,17]. Lastly it may be mentioned that pentacyclic triterpenes with additional cyclic ether have rare natural occurrence and only few references on these are available in literature [21-23].

STRUCTURE ELUCIDATION OF α-AMYRIN (4) β-AMYRIN (5) and β-AMYRIN ACETATE (6)

The fractions eluted from silica gel column with hexane- chloroform (8:2) furnished a mixture of triterpenes. These were purified through thin layer chromatography to yield α- and β- amyrins (**4,5**) and β-amyrin acetate (**6**). Compounds **4** and **5** both displayed [M^+] peaks at m/z 426.3832 in their HRMS mass spectra corresponding to molecular formula $C_{30}H_{50}O$ (calcd. 426.3834) while **6** exhibited molecular ion peak at m/z 468.3945 (calcd. 468.3939). The IR spectrum of **4** & **5** exhibited absorption bands for hydroxyl group (3430 cm^{-1}), and for trisubstituted double bond (3045, 1650 and 815 cm^{-1}), while **6** showed absorptions for trisubstituted double bond (3045,1650,875 cm^{-1}) and acetate group (1710 and 1220 cm^{-1}).

The mass spectrum of **4-6** showed characteristic fragmentation pattern of amyrin skeleton with double bond at C-12 [4-8]. The ^{1}H- and ^{13}C-NMR spectra of **4-6** were in complete agreement with the data reported in literature [4-8].



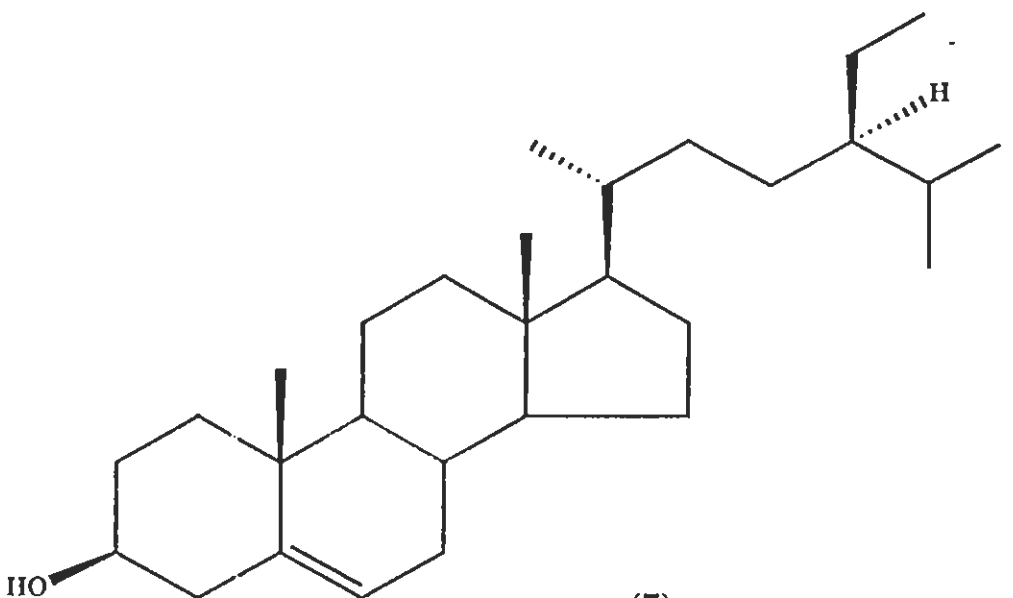
(4) $R^1 = H$, $R^2 = R^3 = CH_3$, $R^4 = H$
(5) $R^1 = R^2 = H$, $R^3 = R^4 = CH_3$
(6) $R^1 = COCH_3$, $R^2 = H$, $R^3 = R^4 = CH_3$

STRUCTURE ELUCIDATION OF β-SITOSTEROL (7)

[24(R)-stigmast-5-en-3β-ol]

7 was obtained as colourless shining crystals with methanol from the fractions eluted with pure chloroform. The high resolution mass spectrum (HRMS) indicated the molecular ion peak at m/z 414.4091 consistent with molecular formula $C_{29}H_{50}O$ (calcd. 414.3990). IR spectrum showed absorption band for hydroxyl group (3450 cm^{-1}) and trisubstituted double bond (3050, 1650 and 815 cm^{-1}).

In the mass spectrum of 7 besides molecular ion peak at m/z 414, the major fragment ion peaks were recorded at m/z 399 $[M-Me]^+$, 396 $[M-H_2O]^+$, 381 $[M-H_2O-Me]^+$. Other abundant ion peaks were observed at m/z 329 $[M-C_7H_5-H_2O]^+$, and 303 $[M-C_7H_9-H_2O]^+$ which were characteristic for sterol with double bond at C-5 [4-8]. The presence of ion peaks at m/z 273 and 255 corresponded to [M-side chain]$^+$ and [M-side chain-H_2O]$^+$, respectively. This mass fragmentation pattern in conjugation with ^{1}H-NMR data showed close identity of 7 to that reported for β- sitosterol [4-8]. It was further confirmed throught co-TLC and mixed melting point with an authentic sample of β- sitosterol.



(7)

EXPERMENTAL

GENERAL NOTES

1.

All melting points were recorded in glass capillary on Gallenkamp melting point apparatus and are uncorrected.

2.

The ultra violet spectra were recorded on Pye Unicam SP-800 spectrophotometer, while infra red spectra were scanned on JASCO A- 302 spectrophotometer.

3.

Electron impact (EI) mass spectra were recorded on Finnigan MAT- 112 and MAT-113 spectrometers coupled with PDP 11/34 computer system. High resolution (HR) mass spectrometry and field desorption (FD) were also performed on MAT-312 mass spectrometer.

4.

^{1}H-NMR spectra were recorded on Bruker AM-300 and AM-400 spectrometers with Aspect 300 data system. The ^{13}C-NMR experiments were performed on the same instruments at 75 and 100-MHz.

5.

Optical rotations were carried out on Schmidt and Haensch polartonic-D polarimeter. Flash column chromatography was performed on Eyela Flash Chromatograph EF-10 model, using silica gel-60 (230- 400 mesh size, E. Merck).

6.

Thin layer chromatography (TLC) was performed on silica gel TLC cards (SIF E. Merck), while column chromatography was performed on silica gel 60 (70-230 mesh, E. Merck).

ISOLATION AND CHARACTERIZATION OF THE CHEMICAL CONSTITUENTS OF SERICOSTOMA PAUCIFLORUM

PLANT MATERIAL:

The plant material (aerial parts, 10 Kg) was collected from the Karachi District and was identified by Plant Taxonomist, Department of Botany, University of Karachi, where a voucher specimen was deposited.

INVESTIGATION OF SERICOSTOMA PAUCIFLORUM

The aerial parts of the freshly collected plant material (10 Kg) were chopped into small pieces and then extracted thrice with methanol (60 L). The combined methanolic extract was evaporated under reduced pressure and the resulting residue was partitioned with water and hexane. The hexane fraction was dried, evaporated, and the resulting residue, which was about 15 g, was then subjected to column chromatography over silica gel (600 g). The elution was successively carried out with increasing polarities of a mixture of hexane and chloroform. The hexane-chloroform eluants (9.5:0.5 and 9:1) yielded crystalline residues which on repeated crystallization from hexane-chloroform provided β- amyrin acetate (**6**) (10 mg) and β- sitosterol (**7**) (18 mg), respectively.

Elution of the column from hexane-chloroform (8:2) was found to be a mixture of triterpenes. It was separated through flash column chromatography over silica gel using solvent system hexane-chloroform (9:1) to obtain α-amyrin (**4**) (10 mg), β-amyrin (**5**) (8 mg), pauciflorinyl acetate (**2**) (18 mg), and pauciflorol acetate (**3**)

(16 mg), respectively.

Another fraction obtained through elution with hexane-chloroform (7:3) was further purified by preparative tlc using solvent system hexane-chloroform (8:2) to provide sericostinyl acetate (1) (22 mg).

CHARACTERIZATION OF SERICOSTINYL ACETATE (1)

Recrystallization with hexane-chloroform provided shining needles of (**1**); m.p. 268-70°; $[\alpha]_D$ -12.34° (c = 0.12, $CHCl_3$).

IR ($CHCl_3$) ν_{max} cm^{-1}: 1710, 1220 (ester group), 1660 (conjugated trisubstituted C = C), and 1325, 1310 (gem- dimethyl group).

UV λ_{max} MeOH nm: 232, 237, 250, 258

EIMS (m/z): (rel.int.%): 466 [$C_{32}H_{50}O_2$, M^+] (90), 451 [$C_{32}H_{50}O_2$- CH_3]$^+$ (20), 423 [$C_{32}H_{50}O_2$-C_3H_7]$^+$ (22), 406 [$C_{32}H_{50}O_2$-$C_2H_4O_2$]$^+$ (18), 391 [$C_{32}H_{50}O_2$-$C_3H_7O_2$]$^+$ (12), 255 [$C_{32}H_{50}O_2$-$C_{13}H_{23}O_2$]$^+$ (100)

^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 6.17 (1H, dd, J = 10.28, and 3.12 Hz, 7-H,) 5.45, (1H, dd, J = 10.47, and 1.72 Hz, 11-H), 4.49 (1H, q, 20- H), 2.04 (3H, s, COMe), 1.01 (3H, s, 25-Me), 0.94 (3H, d, J = 6.58 Hz, 30-Me), 0.92 (3H, s, 26-Me), 0.91 (3H, s, 27-Me), 0.90 (3H, s, 28-Me), 0.89 (3H, s, 24-Me), 0.85 (3H, d, J = 6.10 Hz, 29-Me), 0.72 (3H, s, 23-Me).

^{13}C-NMR ($CDCl_3$, 100.61 MHz) δ: 39.10 (C-1), 18.56 (C-2), 26.46 (C-3), 38.01 (C-4), 55.21 (C-5), 27.95 (C-6), 126.81 (C-7), 131.52 (C-8), 147.03 (C-9), 40.76 (C-10), 123.86 (C-11), 32.91 (C-12), 36.85 (C-13), 33.74 (C-14), 29.71 (C-15), 37.12 (C-16), 43.76 (C-17), 54.12 (C-18), 23.56 (C-19), 80.95 (C-20), 59.30 (C- 21), 40.15 (C-22), 27.82 (C-23), 17.58 (C-24), 26.38 (C-25), 18.19 (C-26), 16.17 (C-27), 17.89 (C-28), 21.22 (C-29), 23.00 (C- 30), 29.57 (H_3C-CO), 170.85 (CO-CH_3).

STRUCTURE ELUCIDATION OF PAUCIFLORINYL ACETATE (2)

Recrystallization with hexane-chloroform provided shining needles of (2); m.p. 240-42° (dec.); $[\alpha]_D$ +47.6° (c = 0.06, $CHCl_3$).

IR ($CHCl_3$) ν_{max} cm^{-1}: 3400-3350 (OH group), 1715, 1210 (ester group), and 1325, 1310 (gem- dimethyl group).

EIMS (m/z): (rel.int.%): 486 [$C_{32}H_{54}O_3$, M^+] (70), 471 [$C_{32}H_{54}O_3$- CH_3]$^+$ (25), 468 [$C_{32}H_{54}O_3$-H_2O]$^+$ (38), 426 [$C_{32}H_{54}O_3$-$C_2H_4O_2$]$^+$ (20), 443 [$C_{32}H_{54}O_3$-C_3H_7]$^+$ (22), 249 [$C_{32}H_{54}O_3$-$C_{16}H_{29}O$]$^+$ (20), 207 [$C_{32}H_{54}O_3$-$C_{18}H_{31}O_2$]$^+$ (45), 189 [$C_{32}H_{54}O_3$-$C_{18}H_{33}O_3$]$^+$ (40).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 4.46 (1H, m, H-20), 3.62 (2H, ABq, J = 13.12 Hz, $C\underline{H}_2OH$), 2.03 (3H, s, $CO\underline{CH}_3$), 1.54 (2H, d, J = 6.58 Hz, H_2-19), 1.21 (1H, m, H-22), 1.17 (1H, m, H-21), 1.02 (3H, s, CH_3- 23), 0.99 (3H, s, CH_3-25), 0.97 (3H, s, CH_3-27), 0.95 (3H, d, J = 7.12 Hz, CH_3-30), 0.84 (3H, d, J = 7.11 Hz, CH_3-29), 0.82 (3H, s, CH_3-28), 0.81 (3H, s, CH_3-26).

^{13}C-NMR ($CDCl_3$, 100.61 MHz) δ: 40.17 (C-1), 18.28 (C-2), 38.82 (C-3), 32.89 (C-4), 55.30 (C-5), 20.21 (C-6), 33.27 (C-7), 41.86 (C-8), 50.45 (C-9), 37.09 (C-10), 23.72 (C-11), 33.27 (C-12), 50.29 (C-13), 41.86 (C-14), 34.59 (C-15), 23.22 (C-16), 55.11 (C-17), 43.39 (C-18), 43.28 (C-19), 81.82 (C-20), 48.75 (C-21), 32.08 (C-22), 28.54 (C-23), 63.14 (C-24), 15.99 (C-25), 16.61 (C- 26), 16.73 (C-27), 15.87 (C-28), 21.32 (C-29), 22.70 (C-30), 171.10 (-O$\underline{C}OCH_3$), 28.02 (CO-$\underline{CH}_3$).

STRUCTURE ELUCIDATION OF PAUCIFLOROL ACETATE (3)

Recrystallization with ether - ethyl acetate provided shining needles of (**3**); m.p. 264-66° (dec.); $[\alpha]_D$ +58.2° (c = 0.12, $CHCl_3$).

IR ($CHCl_3$) ν_{max} cm^{-1}: 1710, 1220 (ester group), 1330, 1310 (gem-dimethyl group), and 1089, 813 (ether group).

EIMS (m/z): (rel.int.%): 484 [$C_{32}H_{52}O_3$, M^+] (62), 469 [$C_{32}H_{52}O_3$-CH_3]$^+$ (24), 441 [$C_{32}H_{52}O_3$-C_3H_7]$^+$ (22), 424 [$C_{32}H_{52}O_3$-$C_2H_4O_2$]$^+$ (20), 409 [$C_{32}H_{52}O_3$-$C_3H_7O_2$]$^+$ (16), 249 [$C_{32}H_{52}O_3$-$C_{16}H_{27}O$]$^+$ (18), 205 [$C_{32}H_{52}O_3$-$C_{18}H_{31}O_2$]$^+$ (40), 189 [$C_{32}H_{52}O_3$-$C_{18}H_{31}O_3$]$^+$ (38).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 4.48 (1H, m, H-20), 3.79 (1H, dd, J = 9.72 and 3.95 Hz, H-3), 3.59 (2H, ABq, J = 12.17 Hz, H_2-25), 2.15 (3H, s, CO$\underline{CH_3}$), 1.52 (2H, d, J = 6.58 Hz, H_2-19), 1.21 (1H, m, H- 22), 1.18 (1H, m, H-21), 0.97 (3H, s, CH_3-27). 0.95 (3H, d, J = 7.12 Hz, CH_3-30), 0.94 (3H, s, CH_3-26), 0.84 (3H, d, J = 7.11 Hz, CH_3-29), 0.83 (3H, s, CH_3-23), 0.81 (3H, s, CH_3-24), 0.80 (3H, s, CH_3-28).

^{13}C-NMR ($CDCl_3$, 100.61 MHz) δ: 40.37 (C-1), 18.81 (C-2), 77.24 (C-3), 33.43 (C-4), 56.18 (C-5), 18.82 (C-6), 34.41 (C-7), 41.90 (C-8), 50.48 (C-9), 37.47 (C-10), 21.0 (C-11), 23.98 (C-12), 49.38 (C-13), 41.82 (C-14), 33.69 (C-15), 22.70 (C-16), 54.70 (C-17), 44.43 (C-18), 43.29 (C-19), 81.82 (C-20), 48.76 (C-21), 32.12 (C-22), 33.51 (C-23), 21.70 (C-24), 63.16 (C-25), 16.59 (C-26), 16.70 (C-27), 15.96 (C-28), 21.64 (C-29), 23.71 (C-30), 171.10 (O$\underline{C}$OCH_3), 23.82 (CO-$\underline{CH_3}$).

CHARACTERISATION OF α-AMYRIN (4)

Crystallization from hexane- chloroform provided colourless needles of (4), m.p. 264-66°; $[\alpha]_D$ +89.5° (c=0.189, $CHCl_3$).

IR ($CHCl_3$) ν_{max} cm^{-1}: 3430 (hydroxyl), 3045, 1650 and 815 cm^{-1} (trisubstituted double bond).

MS (m/z): (rel.intens.%): 426 [$C_{30}H_{50}O$, M^+] (18), 411 [M-Me]$^+$ (15), 408 [M-H_2O]$^+$ (20), 393 [M-Me-H_2O]$^+$ (25), 257 [M-$C_{11}H_{21}O$]$^+$ (21), 218 [M-$C_{14}H_{24}O$]$^+$ (100), 207 [M-$C_{16}H_{27}O$]$^+$ (20), 203 [M- $C_{15}H_{27}O$]$^+$ (45) and 189 [M-$C_{16}H_{29}O$]$^+$ (60).

^{1}H-NMR ($CDCl_3$, 300 MHz): δ 5.11 (1H, m, H-12), 3.19 (1H, dd, $J_{ax,ax}$= 10.0 Hz, $J_{ax,eq}$=4.5 Hz, H-3), 1.08 (3H, s, Me- 27), 1.02 (3H, s, Me-26), 0.96 (3H, s, Me-25), 0.91 (3H, d, J=6.6Hz, Me-30), 1.01 (3H, s, Me-23), 0.81 (6H, s, Me-28) and 0.80 (3H, d, J = 6.8 Hz, Me-29).

^{13}C-NMR ($CDCl_3$, 100 MHz): δ 39.00 (C-1), 27.31 (C- 2), 78.99 (C-3), 39.00 (C-4), 55.23 (C-5), 18.30 (C-6), 33.00 (C- 7), 41.01 (C-8), 47.80 (C-9), 37.00 (C-10), 17.44 (C-11), 124.30 (C-12), 139.34 (C-13), 42.00 (C-14), 28.71 (C-15), 26.62 (C-16), 33.76 (C-17), 59.00 (C-18), 39.61 (C-19), 39.56 (C-20), 31.20 (C- 21), 41.52 (C-22), 28.12 (C-23), 15.62 (C-24), 15.95 (C-25), 1681 (C-26), 23.32 (C-27), 28.00 (C-28), 23.39 (C-29) and 21.34 (C- 30).

CHARACTERISATION OF β-AMYRIN (5)

Crystallization from chloroform-hexane provided colourless shinning needles of β-amyrin (**5**), m.p. 196°; $[\alpha]_D + 100^o$ (c, 0.21, $CHCl_3$).

IR ($CHCl_3$) ν_{max} cm^{-1} 3510 (hydroxyl), 3055, 1635 and 820 cm^{-1} (trisubstituted double bond).

MS (m/z): (rel.intesns. %): 426 [$C_{30}H_{50}O$, M^+] (18), 411 $[M\text{-}Me]^+$ (15), 408 $[M\text{-}H_2O]^+$ (20), 393 $[M\text{-}Me\text{-}H_2O]^+$ (30), 257 $[M\text{-}C_{11}H_{21}O]^+$ (25), 218 $[M\text{-}C_{14}H_{24}O]^+$ (100), 207 $[M\text{-}C_{16}H_{27}O]^+$ (11), 203 $[M\text{-}C_{15}H_{27}O]^+$ (45) and 189 $[M\text{-}C_{16}H_{29}O]^+$ (63).

^{1}H-NMR ($CDCl_3$, 300 MHz): δ 5.23 (1H, m, H-12), 3.21 (1H, dd, $J_{ax,ax}$ = 10.2 Hz, $J_{ax,eq}$ = 4.3 Hz, H-3), 1.15 (3H, s, Me-27), 1.01 (3H, s, Me-26), 0.99 (3H, s, Me-23), 0.95 (3H, s, Me-25), 0.88 (6H, s, Me-29 and Me-30), 0.83 (3H, s, Me-28) and 0.80 (3H, s, Me-24).

^{13}C-NMR ($CDCl_3$, 100 MHz): δ 38.51 (C-1), 27.00 (C- 2), 77.00 (C-3), 37.99 (C-4), 55.10 (C-5), 18.32 (C-6), 32.65 (C- 7), 40.00 (C-8), 47.56 (C-9), 37.00 (C-10), 23.00 (C-11), 122.52 (C-12), 143.50 (C-13), 41.52 (C-14), 28.32 (C-15), 26.23 (C-16), 32.52 (C-17), 47.20 (C-22), 26.01 (C-23), 15.56 (C-24), 15.50 (C- 25), 16.80 (C-26), 26.00 (C-27), 27.32 (C-28), 33.20 (C-29) and 23.69 (C-30).

CHARACTERISATION OF β-AMYRIN ACETATE (6)

β-amyrin acetate (6) was obtained as colourless needles through crystallization with ether-ethyl acetate (yield: 28 mg); m.p. 238-90°; $[\alpha]_D$ +81.4°; (c = 0.21, $CHCl_3$).

IR ($CHCl_3$) ν_{max} cm^{-1}: 3510 (hydroxyl) 3055, 1635 and 820 cm^{-1} (trisub stituted double bond), 1710 and 1220 (acetate group).

MS (m/z): (rel.Intens.%): 468 [$C_{32}H_{52}O_2$, M^+] (24), 408 [M-CH_3COOH]$^+$ (16), 435 [M-Me]$^+$ (15), 257 [M-$C_{13}H_{23}O_2$]$^+$ (25), 218 [M-$C_{16}H_{26}O_2$]$^+$ (100), 203 [M-$C_{17}H_{29}O_2$]$^+$ (45) and 189 [M-$C_{18}H_{31}O_2$]$^+$ (63).

^{1}H-NMR ($CDCl_3$, 300 MHz): δ 5.21 (1H, m, H-12), 3.91 (1H, dd, $J_{ax,ax}$ = 10.2Hz, $J_{ax,eq}$ = 4.3Hz, H-3), 2.01 (3H, s, $\underline{CH_3}$- CO), 1.15 (3H, s, Me-27), 1.01 (3H, s, Me-26), 0.99 (3H, s, Me- 23), 0.95 (3H, s, Me-25), 0.88 (6H, s, Me-29 and Me-30), 0.83 (3H, s, Me-28) and 0.80 (3H, s, Me-24).

^{13}C-NMR ($CDCl_3$, 100 MHz): δ 38.52 (C-1), 27.01 (C- 2), 77.01 (C-3), 37.98 (C-4), 55.14 (C-5), 18.34 (C-6), 32.66 (C- 7), 40.02 (C-8), 47.59 (C-9), 37.02 (C-10), 23.02 (C-11), 122.54 (C-12), 143.52 (C-13), 41.54 (C-14), 28.34 (C-15), 26.26 (C-16), 32.54 (C-17), 47.20 (C-18), 46.81 (C-19), 31.14 (C-20), 34.84 (C- 21), 37.26 (C-22), 26.04 (C-23), 15.57 (C-24), 15.52 (C-25), 16.82 (C-26), 26.06 (C-27), 27.34 (C-28), 33.22 (C-29), 23.68 (C- 30), 21.14 ($\underline{C}H_3$-COO-), and 169.72 (CH_3-$\underline{C}$OO-).

CHARACTERISATION OF β-SITOSTEROL (7)

The chloroform fractions obtained from the column were combined and subjected to flash column chromatography over silica gel. Elution was carried out with solvent gradients of increasing polarities using mixture of ether-chloroform (4:7) and crystallised from methanol (yield: 75 mg): m.p. 135°; $[\alpha]_D$-35.5° (c = 0.1429, $CHCl_3$).

IR ($CHCl_3$) ν_{max} cm^{-1}: 3450 (hydroxyl) 3050 1650 and 815 cm^{-1} (trisubstituted double bond).

MS (m/z): (rel.intens. %): 414 [$C_{29}H_{50}O$, (15), 399 [M-Me]$^+$ (10), 396 [M-H_2O]$^+$ (12), 381 [M-Me-H_2O]$^+$ (72), 329 [M-H_2O-C_5H_7]$^+$ (25), 303 [M-H_2O-C_7O_9]$^+$ (23), 275 [M-H_2O-C_9H_{13}]$^+$ (12), ans 273 [M- $C_{10}H_{21}$]$^+$ (17), 255 [M-$C_{10}H_{21}$-H_2O]$^+$ (30).

^{1}H-NMR ($CDCl_3$, 300 MHz): δ 5.23 (1H, m, H-6), 3.32 (1H, m, H-3), 1.01 (3H, s, Me-19), 0.92 (3H, d, J=6.2 Hz, Me-21), 0.84 (3H, t, J=7.0 Hz, Me-29), 0.83 (3H, d, J=6.5 Hz, Me-26), 0.81 (3H, d, J=6.5 Hz, Me-27) and 0.68 (3H, s, Me-18).

^{13}C-NMR ($CDCl_3$, 75 MHz): δ 37.31 (C-1), 31.81 (C- 2), 71.90 (C-3), 42.40 (C-4), 140.90 (C-5), 121.87 (C-6), 32.07 (C-7), 32.00 (C-8), 50.81 (C-9), 36.61 (C-10), 21.12 (C-11), 40.00 (C-12), 42.61 (C-13), 56.78 (C-14), 24.32 (C-15), 28.24 (C- 16), 56.20 (C-17), 11.90 (C-18), 19.44 (C-19), 36.26 (C-20), 19.10 (C-21), 34.00 (C-22), 29.31 (C-23), 50.36 (C-24), 26.21 (C- 25), 18.80 (C-26), 19.80 (C-27), 23.10 (C-28) and 11.92 (C-29).

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