PHYTOCHEMICAL EVALUATION OF SOME PLANTS USED IN FOLK MEDICINE

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By

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DECLARATION

I hereby declare that this thesis entitled " PHYTOCHEMICAL EVALUATION OF SOME PLANTS USED IN FOLK MEDICINE" is an authentic record of the research work carried out by me under the supervision of Dr.K.K.Vijayan, Professor, Department of Chemistry, University of Calicut, in the partial fulfilment of the requirements for the Degree of Doctor of Philosophy in Chemistry of the University of Calicut, and further that no part thereof has been presented before for any other degree.

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CERTIFICATE

This is to certify that this thesis entitled "PHYTOCHEMICAL EVALUATION OF SOME PLANTS USED IN FOLK MEDICINE" is an authentic record of the research work carried out by Mr.Jose,E.A., under my supervision and guidance in partial fulfilment of the requirements for the Degree of Doctor of Philosophy in Chemistry of the University of Calicut, and further that no part thereof has been presented before for any other degree.

Dr.K.K.Vijayan
(Supervising Teacher)

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PREFACE

The Subject of Phytochemistry is both a demanding and a fast developing field. Phytochemistry deals with the study of secondary metabolites isolated from the plant kingdom, their characterization, reactions, *trans*formations and biological activities. Other areas where phytochemistry has a significant role are agriculture, nutrition and food industry. Plants are sophisticated factories where a variety of chemical compounds are manufactured. These derived products are used as medicines, pesticides, perfumes, fragrances and other utility products. The systematic study of medicinal plants and its claimed use in traditional medicine against a particular ailment not only enables to discover the active substances responsible for that use but also opens interesting avenues for further research.

India has one of the oldest, richest and most diverse cultural traditions associated with the use of medicinal plants. The remarkable fact is that it still remains as a living tradition. Over seven thousand and five hundred species of plants are estimated to be used for human and veterinary health care. Use of plant materials for the treatment of various diseases is very common not only in Third World Countries but even in

developed nations. Nearly all cultures, both ancient and recent, have used plants as source of medicine. We would never be able to say how exactly the ancients discovered the medicinal properties of herbs. Probably, it started with ancient beliefs, myths and lore, got involved with astrology and other occult practices, developed into folk medicine and herbalism, and finally gave rise to traditional systems of herbal medicine. There is at the moment, no exhaustive and reliable inventory available for all the medicinal plants of India.

The use of plants in curing and healing is as old as man himself. One of the earliest known records of medicinal therapy is the Ebers Papyrus, which is thought to have been written in 1550 B.C. (Egyptian period). Among many agents used in at that time, there are some which are still employed in medicine, such as opium and castor oil.

Plant kingdom, nature's gift to man, has attended to all his basic needs namely, food, clothing, shelter and also medicine. A vast knowledge of medicinal plants must have accumulated, especially in the tropics, where the large majority of all higher plant species are found. In tropical developing countries about 80% of the rural population depend for their health care on traditional systems of medicine, which are by and large based on plant products. Many potent drugs are derived directly from plants or processed from chemicals obtained therefrom. This fact, together with the WHO programme launched in 1976 for the 'Promotion

and Development of Traditional Medicine' should provide strong reasons for devoting considerable resources to research on medicinal plants in the tropics.

India is a large thickly populated country of the developing world. Among many unique social and cultural features, India enjoys the significance of its rich heritage in the field of ancient medicine. Probably, India is one of the countries where traditional medicine is most developed and is an official system of medicine. In India, ancient Indian medicine is maintaining almost a parallel structure to western modern medicine and as such is responsible for health care delivery to the major proportion of the population especially in rural areas and also in urban areas.

The age old practice of using extractives of plants for the treatment of various diseases is well known and forms the basis of the system of Ayurvedic and folk medicines. However, due to the lack of proper scientific base these systems of medicines have not developed as fast as the modern system, in spite of the fact that the efficacy of certain herbal medicines has been proved beyond doubt.

Ayurveda, the Indian system of medicine which make use of plant extractives, was developed more than three thousand years ago. Phytochemical studies have increased the credibility of Ayurvedic system of treatment. But in the developed world, the use of plants in medicine is

often looked upon as obsolete and largely based on superstition. Scientists who are working in the field of so called modern biology, like molecular biology and biotechnology, seem to have little understanding of the research need in this field. There are also strong antagonists to research programmes on medicinal plants among the de*cis*ion makers and funding agencies. Thus, foreign invasions and mystic practices that existed in this traditional system reduced it into a folk-lore.

Though Allopathy has succeeded in making several drugs synthetically, many of the man made substitutes for plant substances are not considered fully satisfactory. In fact, there is a revival of interest in natural products as these are biologically more compatible with human systems and comparatively less toxic than the synthetics. Recently several European countries have bestowed much interest in Ayurveda, folk medicines and other oriental practices. According to Shah (1982) 50 percent of the prescriptions in Europe contained natural products or their constituents. Also, the time and cost involved for the production of a new drug are quite high. In most of the cases, the original source is cheaper, more in abundance and better in quality. Many of the chemicals from plants are unique, produced through complex chemical reactions, which are difficult or impossible to duplicate with. As a matter of fact, the isolation and characterization of the active principles of some crude drugs such as Ephidrine from 'Ma Huang', Reserpine from Rauwolfia,

Morphine and related compounds from Opium, Vincristine from Vinca rosea, and recently Taraxerol from Hibiscus, for examples, once again attracted the attention of outside world into this traditional system of medicine. Now research works are going on to isolate plant ingredients which can cure various human ailments. Scientists are now in search of drugs which can cure Cancer, Anthrax and AIDS, the fatal diseases of the present.

After a period of disregard and decline, these traditional systems of 'green medicine' are one again, back to the centre-stage of our health-care programmes. There has been a steady increase in demand for such medicines and these systems have now regained respectability among the scientific community, the world over. Fairly high percentage of useful plant derived drugs were discovered as a result of scientific follow - up of well- known plants used in traditional medicine and hence this is a good approach for discovering other useful drugs from plant sources.

The revolutionary advances in the field of chromatography and spectroscopy have made isolation and structural elucidation more simple and reliable. Hence, it is of tremendous importance for us to study the medicinal plants used in folk medicine using modern scientific techniques to evaluate its use in the fight against diseases.

In this work, two plants, ***Hibiscus Schizopetalus*** of Malvaceae family and ***Tabernaemontana alternifolia*** of Apocynaceae family have been investigated for their chemical constituents. These plants have been recognized as medicinal plants in folk medicine and so these form an ingredient in many of the Folk and Ayurvedic preparations.

The work done is presented in seven chapters:-

Chapter I has two sections. In **Section 1** a review on Phytochemical studies of Hibiscus and in **Section 2** a general introduction to triterpenoids, an important secondary metabolites of Hibiscus species, are given.

Chapter II to V deal with the phytochemical investigation and characterization of five compounds isolated from *H.schizopetalus*.

In **Chapter II** an introduction to the plant *H.schizopetalus* and different compounds isolated from the petroleum ether extract of leaves of *H.schizopetalus* are given. Also the characterization of first compound **H-1**, *cis*-Hentriacont-3-ene, is discussed.

In **Chapter III** characterization of the second and third fractions, **H-2** and **H-3**, are described. The Compound **H-2** is identified as 22-Hydroxytaraxeryl acetate. The fraction **H-3** is found to be a mixture of 22-Hydroxytaraxeryl acetate **(H-3a)** and 22-Hydroxytaraxeryl-*Cis*-p-coumarate **(H-3b)**.

In **Chapter IV** the characterization and structure elucidation of the fifth fraction **H-5** is described instead of **H-4**. This change in the elution order is brought because of the structural similarity of the compound **H-5** with the compound **(H-3b)**. The Compound **H-5** is identified as 16-Hydroxytaraxeryl –*trans*-p-coumarate.

In **Chapter V** the characterization of fourth fraction **H-4** is discussed. The compound **H-4** is characterized as 7,24-Dihydroxylanosta-22,25-dien-3β-ol-*trans*-p-coumarate.

In **Chapter VI**, the general experimental procedures adopted for the isolation and characterization of compounds isolated from the leaves of *H.schizopetalus* are described.

Chapter VII is about the phytochemical studies on *Tabernaemontana alternifolia*. This chapter has two sections. In **Section I**, the phytochemical studies already done in the Tabernaemontana species are described. In **Section 2**, isolation and characterization of compounds from red pulpy seed coating of *T.alternifolia* and experimental procedures adopted are discussed.

References are given after Chapter VII in serial order. **Spectra** of all compounds isolated and characterized are appended at the end of the thesis.

As far as possible the tables, schemes and figures are given soon after their mention in the thesis.

Section 1

Phytochemical studies on Hibiscus species - A review

Section 2

The Triterpenoids- An Important Secondary Metabolite of Hibiscus species.

Jose E.A " Phytochemical evaluation of some plants used in folk medicine" Thesis. Department of Chemistry , University of Calicut, 2002

CHAPTER – I

Section - 1

PHYTOCHEMICAL STUDIES ON HIBISCUS SPECIES - A REVIEW

Introduction

Hibiscus is one of the genera of the large family, Malvaceae. It includes about 100 genera and more than 2000 species. They are found in tropics and sub tropics as herbs or shrubs, rarely trees, most of them having showy flowers. Leaves are alternate, stipulate, simple and sometimes palmately lobed. Calyx is 5-toothed. Flowers are bisexual pentamerous, mostly bell shaped, axillary, solitary, often very large and complete. About 160 species of this genera are identified. Some members of this genera are reported to be medicinally as well as economically important. Also there are many instances of using Hibiscus plants in folk medicine. A few are yielding food and fibre products.

Medicinally, the different parts of the plants of Hibiscus genus and the infusion, decoction etc., derived from them are used for many

therapeutic purposes. Seeds of *H. esculentus, H. manihot, H.sabdariffa* etc. yield edible oils. The fatty oils obtained from *H. cannabinus* is used for the manufacture of soaps and varnishes. Seed oils of some species like *H. abelmoschus* also known as Musk seed oil or Ambrette seed oil are used in perfumery. Many members of this genus are used for the manufacture of fibers. *H. esculentus* gives a representative mucilage called Okra mucilage which have better sustaining characteristics than even widely used synthetic materials like methyl cellulose[1]. Seeds of *H. sabdariffa* which is widely cultivated in India have remarkable coagulating property when applied to turbid water[2].

Some species are reported to be medicinally important[3,4]. Infusion, decoction etc. derived from some of them are used for many therapeutic purposes. Various parts of the plant, *H. mutabilis*, commonly called Guliajaib, are used medicinally. Leaves, for example, is used for cough, menorrhagia and wounds caused by burns and scalds. A mucilaginous preparation from the fruits of *Hibiscus esculentus* has found application as a plasma replacement or blood volume expander. Another member, *H. rosa-sinensis*, commonly known as China rose plant, appears to have potent contraceptive effect. In Ayurvedic literature, the flowers of this plant are attributed to possess antifertility effect[5]. Ether soluble portion of the water insoluble fraction of the benzene extract showed the most

significant anti-implantation and abortifacient activity[6-9]. Flowers are not found to have any such side effects as would warrant its withdrawal. This is a welcome feature considering the well-known side effects of currently available contraceptives which are mostly hormonal in nature. A number of compounds like cyanidin, quercetin, hentriacontane, cyanidin diglucoside, calcium oxalate, tannins, thiamine, riboflavin, niacin and ascorbic acid have been reported from this plant. But none has found to have antifertility activity. The leaves, flowers, fruits and seeds of various Hibiscus species are of definite food value.

H. sabdariffa calyx is reported to have antispasmodic potential[10]. This plant is also studied for its estrogen like activity[11]. Rats fed on *H. sabdariffa* oil showed inferior growth and reproductive performances. *H. sabdariffa* also shows antimicrobial activity against many bacteria[12]. The seed of *Hibiscus abelmoschus* is also called Viper seed or Snake seed, since it is used to cure snake bite. The seed contained no alkaloid and no glycoside, but had about 43% adsorbent capacity of that of activated charcoal[13]. The action of Viper seed may be that of finely dispersed emulsions which inactivated cobra venom by adsorption. Chemical analysis of the seed gave the following results by weight. Moisture 11.4%, ash 5.3%, starch 13.35%, protein 2.3%, crude fibre 31.46% and oil 14.5%. Leaves of *H. esculentus* was shown to have

antibiotic action [14] against *Escherichia coli* and the bark of *H. syriacus* showed antifungal activity[15].

Malvaceae family as a whole and Hibiscus genus in particular, are rich sources of secondary metabolites of varying categories. The chemical as well as the pharmacological importance of some of the compounds isolated from Hibiscus species tempted chemists and pharmacologists to investigate further on these plants. Most of the phytochemical works in various Hibiscus species are concentrated on flowers. Studies on leaves, root bark etc. are less. The important classes of compounds isolated from them are described in the following paragraphs.

Flavonoids

Flavonoids are a group of natural plant pigments. Structurally they are Benzpyrone pigments. Numerous flavonoids have been isolated from various plants belonging to Hibiscus genus of the Malvaceae family. Most of the flavonoids isolated from Hibiscus species are glycosides of quercetin, kaempferol and gossypetin. Some flavonoids like gossypin, quercetin-3-robinobioside, sabdaretin and hyperin are of pharmacological interest. The decoction of the flower extract of *Hibiscus sabdariffa* containing several flavonoids like sabdaretin, gossypin etc. acts as a hypotensive agent.[16] Quercetin-3-robinobioside, quercetin-3-glucoside, hyperin, quercetin and myricetin were isolated from *Abelmoschus*

manihot (*H. manihot*), flowers of which are used in the treatment of chronic bronchitis and tooth ache[17]. A new flavanone glycoside, Naringenin-5,7-dimethylether-4'-O-β-D-xylopyranosyl-β-D arabinopyranoside in addition to various flavonoids like isoquercitrin and quercetin-3-sambubioside have been isolated from *H. mutabilis,* which is used medicinally[18]. Flavonoids from various Hibiscus species are given in **Table 1.1.**

Anthocyanins

Anthocyanins are another group of pigments based on benzopyrilium or flavylium chloride structure. These pigments are responsible for the intense colour of flowers, leaves and fruits of various plants. Numerous shades of colours are due to same skeletal structure, but different substitution. A number of anthocyanins have been isolated from the flowers and leaves of various Hibiscus species. Most of them are glycosides of cyanidin and delphinidin. Three new anthocyanidin malonylglycosides have been reported from *H. syriacus.*[19], along with the known compounds like 3-0-glucosides of delphinidin, cyanidin, petunidin, malvidin and three known anthocyanidin 3-0-malonylglucosides.[20] Most common anthocyanin is cyanidin-3-sambubioside. Cyanidin -3-glucoside is found in large woody species and cyanidin-3-sophoroside is present mostly in plants like

H. rosa-sinensis i.e. in the section Lilibiscus of Malvaceae family. Anthocyanins and anthocyanidins reported from various Hibiscus species are given in **Table 1.2.**

Terpenoids and steroids

β-Sitosterol, the commonly occurring plant sterol has been isolated from various members of this genus. The seed oil of *H. sabdariffa* is found to contain ergosterol, the most important mycosterol. The other sterols isolated from it are cholesterol, campesterol, stigmasterol, β-sitosterol and α-spinasterol[21]. Several monoterpenes like limonene, citral and phellandrene are isolated from the seed oil of *H. cannabinus*[22]. Triterpenoids like taraxeryl acetate are isolated from some members like *H. rosa-sinensis.* Several sesquiterpenoid quinones and related compounds (hibiscones A-D and hibiscoquinones A-D) are isolated from some other members[23]. The petroleum ether extract of the leaves of *H. furcatus* yielded five components. They were identified to be friedelin, β-sitosterol, taraxerol, a mixture of n-alkanes and mixture of n - alkanols by chemical and physical methods[24]. Analysis of volatile oils of *H. esculentus* indicated the presence of about 148 compounds of which 16 were terpenes[25]. The terpenoids and steroids isolated from Hibiscus species are tabulated in **Table 1.3.**

Sugars

Sugars may be present either in the bonded state i.e. with hydroxyl groups present in the aglycones of flavonoids and anthocyanins or in the free state. The most common sugars are glucose, galactose, xylose, rhamnose and sucrose. Although glycosides like robinobioside, sophoroside and sambubioside have been reported from different Hibiscus species, free sugars robinobiose, sambubiose and sophorose haven't been reported from Hibiscus species. Sugars isolated from Hibiscus species are listed in **Table 1.4.**

Amino acids

Various amino acids are isolated from different species of Hibiscus genus of Malvaceae family. Recently, thiamine, riboflavin and niacin are isolated from *H. rosa-sinensis*. Amino acids isolated form this genus are given in **Table 1.5.**

Fatty acids and related compounds

Fatty acids occur as glycerides in oils and fats and combined with flavonoids, anthocyanins and leucoanthocyanins as their esters. There is a co-occurrence of cyclopropene acids and epoxy acids in the seed oils of Malvaceae (especially in the genus Hibiscus). Studies on the fatty acid constituents of Hibiscus have been mainly confined to seed oils in which

palmitic, stearic, oleic and linoleic acids are the main compounds [26-28]. Citric acid, malic acid and tartaric acid occur in free state in plants like *H. sabdariffa*. The infusion of *H. sabdariffa* is a refreshing beverage. It has diuretic and chloretic effect, decreases the viscosity of blood and reduces blood pressure. It also reduces the intestinal peristalsis[29]. The stem bark of *H. rosa-sinensis* yielded lettuce seed germination inhibitors. The active constituents were four fatty acid methyl esters, 9-decynoate, 8-nonynoate, 10-oxo-11-octadecynoate and 8-oxo-9-octadecynoate. The other two compounds isolated were, methyl-10-methylene-9-oxo-octadecanoate and methyl-9-methylene-8-oxo-heptadecanoate[30,31]. They are one of a few classes of aliphatic compounds possessing a terminal acetylenic linkage from higher plants. Two novel cycloprene acids sterulic and malvalic acids are isolated from the seeds of *H. esculentus*[32]. Two acetylenic compounds stearolic (9-octadecynoic) and 8-heptadecynoic acids are isolated from the seeds of *H. syriacus*. Two novel aliphatic enone ethers, methyl (E)-11-methoxy-9-oxo-nonadecenoate and (E)-10-methoxy-8-oxo-9-octadecenoate were also isolated from the stem bark of *H. rosa-sinensis*[33]. Various fatty acids and their derivatives isolated from Hibiscus genus are given in **Table 1.6.**

Alcohols and Hydrocarbons

A large number of alcohols and hydrocarbons have been isolated from various members of this genus. They are listed in **Table 1.7** and **Table 1.8**. respectively.

Miscellaneous compounds.

In addition to the compounds mentioned above, several other classes of compounds have been isolated from many members of this genus. They include amines[34,35], carbohydrates[36], carotenes[37], catechols[38,39], pentosans[40], phospholipids[41,42,43], polyphenols[44], polyuronides[45], ascorbic acid, proteins[36], proanthocyanidins[46,47] etc. The mucilage obtained from certain members like *H.syriacus*[48], *H.esculentus*[49,50] are also studied.

Table 1.1. Flavonoids of Hibiscus species

No	Name	Source	Reference
1.	Cannabiscitrin	*H. cannabinus*	51
2.	Gossypetin	*H. sabdariffa* *H.tiliaceus* *H. suratensis*	52, 53, 54
3.	Gossypin	*H. esculentus* *H. sabdariffa* *H. furcatus*	55, 56, 57, 58

4.	Gossypitrin	*H. sabdariffa* *H. suratensis* *H. furcatus*	52, 54, 58, 59
5.	Gossytrin	*H. sabdariffa*	60
6.	Gossypetin-8-(rhamnosyl glucoside)	*H. esculentus*	56
7.	Hibifolin	*H. vitifolius*	73
8.	Hibridin	Hibrid Hibiscus	74
9.	Hibiscatin	*H. furcatus*	58
10.	Hibiscetin	*H. sabdariffa* *H. esculentus* *H. vitifolius*	52
11.	Isoquircitrin	*H. cannabinus* *H. mutabilis*	61, 62
12.	Kaempferol	*H. tiliaceus*	53
13.	Kaempferol-3-glucoside	*H. cannabinus*	61
14.	Kaempferol-3-rhamnoside	*H. cannabinus*	61
15.	Kaempferol-3-galactoside	*H. tiliaceus*	63
16.	Kaempferol-3-rhamnosyl glucoside	*H. rosa-sinensis*	61
17.	Kaempferol-3-β-rhamnopyranosido-7-∝-rhamnofuranoside	*H. cannabinus*	64
18.	Kaempferol-7-∝-L-rhamnopyranoside	*H. cannabinus*	65
19.	Kaempferol-3-xylosylglucoside	*H. rosa-sinensis*	57

20.	Quercetin	Various Hibiscus species	52, 53, 65 66,67
21.	Quercetin-3-β-D-glucopyranoside	108F cotton-Hibiscus hybrid	68
22.	Quercetin-3-D-galactoside	*H. tiliaceus*	63
23.	Quercetin-3-D-glucosylglucoside	*H. esculentus*	56
24.	Quercetin-3-robinobioside	*H. manihot*	17
25.	Quercetin-3-sambubioside	*H. mutabilis*	69
26.	Quercetin-3-4'-(diglucosyl glucoside)	*H. esculentus*	56
27.	Quercetin-3-7-diglucoside	*H. rosa-sinensis*	57
28.	Quercetin-3'-glucoside	*H. manihot*	17
29.	Quercetin-4'-glucoside	*H. esculentus* *H. mutabilis*	56, 62
30.	Quercetin-5-glucoside	*H. esculentus*	56
31.	Quercetin-5-rhamnosyl glucoside	*H. esculentus*	56
32.	Quercimeritrin	*H. esculentus* *H. mutabilis*	56, 70
33.	Myricetin	*H. sabdariffa* *H. manihot*	62
34.	Myricetin-3'-glucoside	*H. abelmoschus*	71
35.	Rutin	*H. cannabinus* *H. mutabilis*	61, 64
36.	Sabdaretin	*H. sabdariffa*	52
37.	Saponarin	*H. syriacus*	72

Table 1.2. Anthocyanins of Hibiscus species

No	Name	Source	Reference
1.	Cyanin	*H. rosa-sinensis* *H. mutabilis*	57, 62, 69, 75
2.	Cyanidin	*H. rosa-sinensis* *H. mutabilis*	54, 67, 76, 77
3.	Chrysanthamin	Various Hibiscus species	66, 78, 79, 80, 81, 82, 83
4.	Cannabinin	*H. cannabinus*	81, 84, 43
5.	Cyanidin-3- sambubioside	*H. sabdariffa*	82,85
6	Cyanidin -3-sophoroside	Various Hibiscus species	83, 85, 86
7	Cyanidin -3- (2-glucosyl-glucoside)	*H. sabdariffa*	57
8	Cyanidin -3- sophoroside-5-glucoside	*H. rosa-sinensis*	57
9	Cyanidin -3-,4'- diglucoside	*H. esculentus*	56
10	Cyanidin -3- rutinoside-5-glucoside	*H. mutabilis*	62
11	Cyanidin -4'-glucoside	*H. esculentus*	56
12	Delphinidin	*H. sabdariffa*	78
13	Delphinidin-3-glucoside	*H. sabdariffa*	78
14	Delphinidin-3-sambubioside	*H. sabdariffa*	82,85
15	Gossypicyanin	Hibrid Hibiscus	79, 81, 87
16	Hirsutine	Hybrid Hibisucs with cotton tree	88
17	Ilicicyanin	*H.mutabilis*	66
18	Mytrillin	*H. cannabinus*	43

Table 1.3. Steroids and Terpenoids of Hibiscus species.

No	Name	Source	Reference
1.	Campesterol	*H. sabdariffa*	89
2.	Cholesterol	*H. sabdariffa*	89
3.	Ergosterol	*H. sabdariffa*	89
4.	β- Sitosterol	Various Hibiscus species	24, 61, 63, 90, 91, 92, 93, 94
5.	β- Sitosterol-β-D-glucoside	*H. abelmoschus*	92
6.	β- Sitosterol-β- D-galactopyranoside	*H. sabdariffa*	95, 96
7.	Stigmasterol	*H. sabdariffa*	89
8.	∝-Spinasterol	*H. sabdariffa*	89
9.	Limonene	*H. cannabinus*	22
10.	Citral	*H. cannabinus*	22
11.	Phellandrene	*H. cannabinus*	22
12.	∝-terpenylacetate	*H. cannabinus*	22,93
13.	Taraxerol	*H. furcatus*	24
14.	Taraxeryl acetate	*H. rosa-sinensis* *H. esculentus*	91
15.	Friedelin	*H. furcatus*	24

Table 1.4. Sugars of Hibiscus species

No	Name	Source	Reference
1.	D-glucose	Various Hibiscus species	22,93, 97, 98, 99, 100, 101, 102
2.	Xylose	*H. abelmoschus* *H. ficulneus*	97, 98, 99, 100, 101, 103
3.	L-arabinose	various Hibiscus species	97, 98, 99, 100, 101, 103
4.	L-rhamnose	various Hibiscus species	79, 97, 99, 100, 103
5.	Stachyose	*H. cannabinus*	104
6.	Raffinose	*H. cannabinus*	104
7.	Sucrose	*H. esculentus* *H. cannabinus*	22, 93, 104
8.	D-galactose	*H. esculentus* *H. ficulneus*	93, 97, 98, 99
9.	Mannose	*H.esculentus,* *H. cannabinus*	22,93
10	Lactose	*H.esculentus,* *H. cannabinus*	22,93
11	Fructose	*H. cannabinus*	22,93

Table 1.5. Amino acids of Hibiscus species

No	Name	Source	Reference
1.	Alanine	*H. cannabinus*	96
2.	∝-Amino adipic acid	*Various Hibiscus species*	105
3.	Arginine	*H. esculentus, H. cannabinus*	96, 106
4.	Aspartic acid	*H. sabdariffa, H. cannabinus*	96, 107
5.	Cystine	*H. sabdariffa H. cannabinus*	96
6.	Glutamic acid	*H. sabdariffa H. cannabinus*	96
7.	Glycine	*H. cannabinus*	96
8.	Histidine	*H. cannabinus H. esculentus*	96
9.	Homoserine	*Various Hibiscus species*	105
10.	Isoleucine	*Various Hibiscus species*	96
11.	Leucine	*Various Hibiscus species*	96
12.	Lysine	Various Hibiscus species	96
13.	Methionine	Various Hibiscus species	96
14.	Phenylalanine	*H. esculentus*	106
15.	Proline	*H. cannabinus*	96
16.	Threonine	*H. esculentus*	106
17.	Tryptophan	*H. esculentus H. cannabinus*	96, 106
18.	Tryosine	*H. cannabinus*	96
19.	Valine	*H. esculentus*	106

Table 1.6. Organic acids of Hibiscus species

No	Name	Source	Reference
1.	Arachidic acid	*H. esculentus* *H. rosa-sinensis*	90, 93
2.	Ascorbic acid	*H. sabdariffa*	108
3.	Citric acid	*H. sabdariffa* *H. rosa-sinensis*	109, 110
4.	Cerotic acid	*H. sabdariffa*	89
5.	p-coumaric acid	*H. rosa-sinensis* *H. tiliaceus*	63, 111
6.	Fumaric acid	*H. rosa-sinensis* *H. tiliaceus*	63
7.	Hibiscus acid	*H. sabdariffa*	112, 113
8.	Hydroxy citric acid	*H. furcatus* *H. cannabinus*	114, 115
9.	Hexadecanoic acid	*H. esculentus*	116
10.	Linoleic acid	*H. esculentus* *H. cannabinus*	61, 116
11.	Lignoceric acid	*H. cannabinus*	89
12.	Malic acid	*H. sabdariffa*	109
13.	Malvalic acid	*H. rosa-sinensis* *H. caesius*	117, 118
14.	Oleic acid	*H. esculentus*	116
15.	12, 13 - epoxy oleic acid	*H. esculentus*	116
16.	Oxalic acid	*H. rosa-sinensis* *H. sabdariffa*	119
17.	Palmitic acid	*Various* *Hibiscus species*	90, 93, 114, 116
18.	Stearic acid	*Various* *Hibiscus species*	90, 93, 114, 116
19.	Sterulic acid	*H. rosa-sinensis* *H. caesius*	111, 117, 118
20.	Tartaric acid	*H. sabdariffa* *H. rosa-sinensis*	109, 119

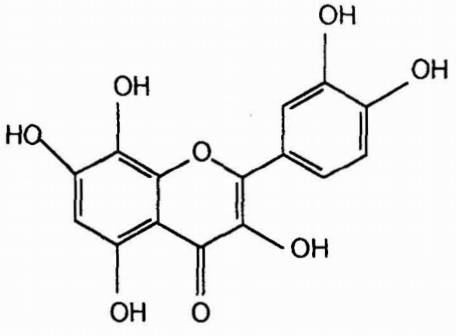
Table 1.7. Alcohols of Hibiscus species

No	Name	Source	Reference
1.	Methyl alcohol	*H. esculentus*	93
2.	Ethyl alcohol	*H. esculentus* *H. cannabinus*	22,93
3.	Isopropryl alcohol	*H. esculentus*	93
4.	3-methyl-1-butanol	*H. esculentus*	93
5.	Cyclohexanol	*H. esculentus*	22,93
6.	Hexacosanol	*H. canabinus*, Hybrid Hibiscus	89, 110
7.	Octacosanol	Hybrid Hibiscus	110
8.	Hexadecanol	Hybrid Hibiscus	110
9.	Octadecanol	Hybrid Hibiscus	110
10.	Triacontanol	*H. cannabinus*	89
11.	Dotriacontanol	*H. cannabinus*	89

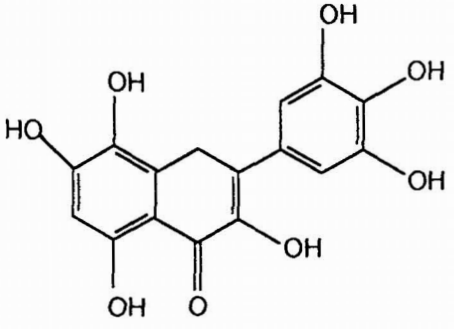
Table 1.8. Hydrocarbons of Hibiscus species

No	Name	Source	Reference
1.	Hexacosane	*H. cannabinus*	89
2.	Heptacosane	*H. cannabinus*	89
3.	Octacosane	*H. cannabinus*	89
4.	Nonacosane	*H. cannabinus*	89
5.	Hentriacontane	*H. cannabinus*, Hybrid Hibiscus	89, 110
6.	Dotriacotane	*H. cannabinus*, Hybrid Hibiscus	89, 110
7.	Tritriacontane	*H. cannabinus*, Hybrid Hibiscus	89, 110
8.	Tetratriacontane	*H. cannabinus*, Hybrid Hibiscus	89, 110
9.	Pentatriacontane	*H. cannabinus*, Hybrid Hibiscus	89, 110
10.	Hexatriacontane	*H. cannabinus*, Hybrid Hibiscus	89, 110
11.	Heptatriacontane	*H. cannabinus*, Hybrid Hibiscus	89, 110
12.	Octatriacontane	*H. cannabinus*, Hybrid Hibiscus	89, 110
13.	Tetracontane	*H. cannabnus*	89

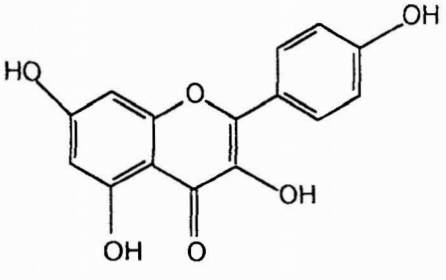
Structures of some important compounds reported from Hibiscus species



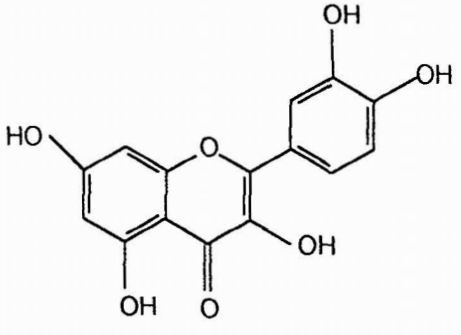
Gossypetin



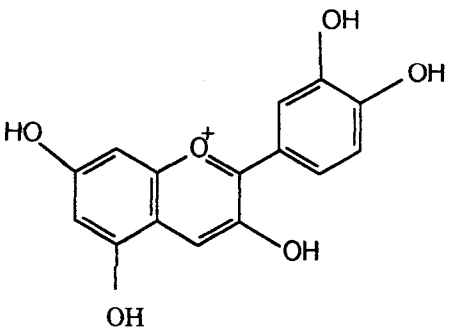
Hibiscetin



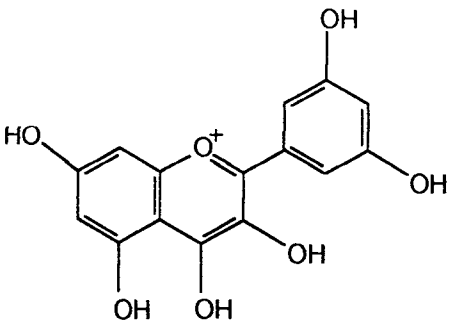
Kaempferol



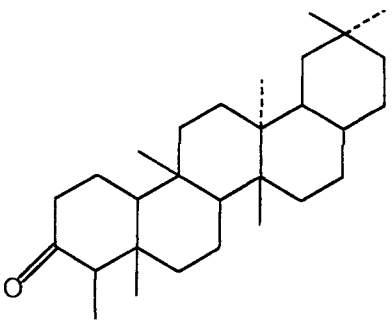
Quercetin



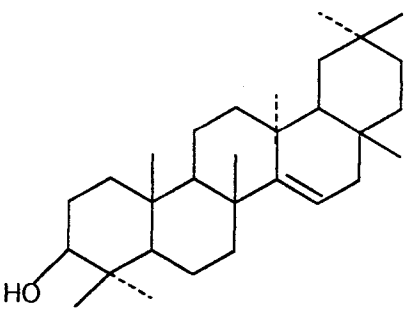
Cyanidin



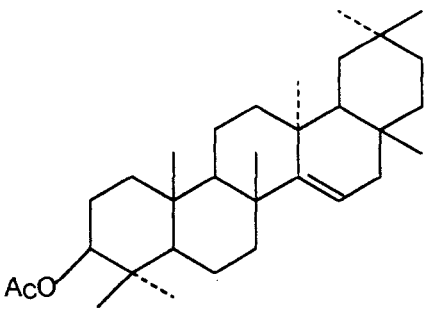
Delphinidin



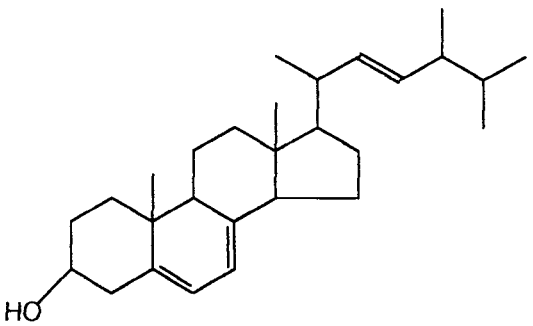
Friedelin



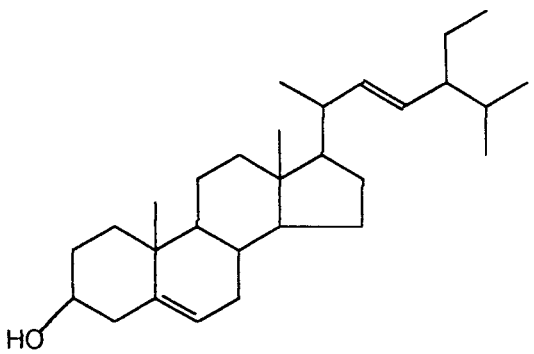
Taraxerol



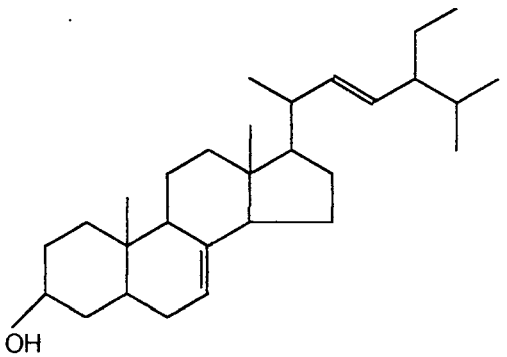
Taraxeryl acetate



Ergosterol



Stigmasterol



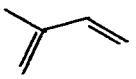
α-Spinasterol

Section 2

THE TRITERPENOIDS - AN IMPORTANT SECONDARY METABOLITE OF HIBISCUS SPECIES.

The Terpenoids are one of the important class of secondary metabolites isolated from Hibiscus species. Very vital pharmacological activities are shown by these compounds. Also in the phytochemical examination of *H. schizopetalus*, the present work, the main isolates are pentacyclic trierpenoids. Consideration of the importance of this class of compounds, the following account is presented which describes the various significance of cyclic triterpenes .

The terpenoids are amongst the most widespread and chemically interesting groups of natural product. However, despite their structural diversity, they have a simple unifying feature which we shall use as their definition. Terpenoid compounds may be defined as a group of natural product whose structure may be divided into isoprene units,



They are classified according to whether they contain two (C_{10}), three (C_{15}), four (C_{20}), five (C_{25}), six (C_{30}) or eight (C_{40}) such units. Accordingly the different class of compounds are monoterpenes, sesquiterpenes,

diterpenes, sesterterpenes, triterpenes, carotenoids and polyterpenoids, $(C_5)_n$.

They range from the essential oil components, the volatile mono - and sesquiterpenes (C_{10} and C_{15}), through the less volatile diterpenes (C_{20}) to the involatile triterpenoids and Sterols (C_{30}) and Carotinoid pigments (C_{40}). Each of these various classes of terpenoid are of significance in either plant growth, metabolism or ecology.

Chemically, terpenoids are generally lipid soluble. The terpenoids are normally extracted from plant tissues with light petroleum, ether or chloroform and can be separated by chromatography on silica gel or alumina using the same solvents. There is, however, often difficulty in detection on a microscale, since all (except carotenoids) are colourless and there is no sensitive universal chromogenic reagent for them. Reliance often has to be placed on relatively non-specific detection on TLC plates with Con. H_2SO_4 and heating.

Isomerism is common among terpenoids and pairs of isomeric forms may be isolated from plants; one such pair are the monoterpenes geraniol and nerol. In addition, terpenoids are mostly alicyclic compounds and because the cyclohexane ring is usually twisted in the so-called chair form, different geometric conformations are possible, depending on the substitution around the ring. The stereochemistry of the

cyclic terpenoids is therefore highly involved and often difficult to determine.

A considerable number of quite different functions have been ascribed to plant terpenoids. Their growth regulating properties are very well documented; two of the major classes of growth regulators are the sesquiterpenoid abscisins and the diterpenoid-based gibberellins. Astringent and toxic properties of sesquiterpenoid and diterpenoid bitter principles figure in many folk medicines. The important contribution of carotenoids to plant colour is well known and it is almost certain that these C_{40} terpenoids are also involved as accessory pigments in photosynthesis. The importance of mono and sesquiterpenes in providing plants with many of their distinctive smells and odours is also familiar to most scientists. Less is generally known of the role of terpenoids in the more subtle interactions between plants and animals, e.g. as agents of communication and defence among insects, but this is now an area of active research. Finally, it should be mentioned that certain non-volatile terpenoids have been implicated as sex hormones among the fungi.

The triterpenoids

The terpenoids form a very large group of naturally occurring substances, widely distributed throughout the plant kingdom. They are having a carbon skeleton based on six isoprene units and which are

derived biosynthetically from the acyclic C_{30} hydrocarbon, squalene. They have relatively complex cyclic structures, most being either alcohols, aldehydes or carboxylic acids. They are colourless, crystalline, often high melting, optically active substances, which are generally difficult to characterize because of their lack of chemical reactivity. A widely used test is the Liebermann-Burchard colour reaction, which produces a blue-green colour with most triterpines and sterols. Although the isolation of many well-known triterpenoids dates back to the last century, the first correct structures were not assigned until the time of the second World War. Thus the parent substances β-amyrin, α-amyrin, and lupeol were correctly formulated respectively in 1937, 1949 and 1951.[120,121]

Triterpenoids can be divided into at least four groups of compounds: true triterpenes, steroids, saponins and cardiac glycosides. The latter two groups are essentially triterpenes or steroids which occur mainly as glycosides.

Triterpenoids together with steroids have played an important part in laying the foundation of the "New Organic Chemistry". In particular they provided an excellent experimental basis for the principles of conformational analysis [122,123].

Many Triterpenes are known in plants and new ones are regularly being discovered and characterized[124]. So far only a few are known to be of wide spread distribution. This is true of the pentacyclic triterpenes α- and β- amyrin and the derived acids, ursolic and oleanolic acids. These and related compounds occur especially in the waxy coatings of leaves and on fruits such as apple and pear and they may serve a protective function in repelling insect and microbial attack. Triterpenes are also found in resins and barks of trees and in latex.

Certain triterpenes are notable for their taste properties, particularly their bitterness. Limonin, the lipid soluble bitter principles of citrus fruits, is a case in point. It belongs to series of pentacyclic triterpenes which are bitter, known as limonoids and quassinoids. They occur principally in the Rutaceae, Meliaceae and Simaroubaceae[125] and are also of chemotaxonomic interest[126]. Another group of bitter triterpenes are the cucurbitacins, confined mainly to the seed of various cucurbitaceae but recently detected also in the cruciferae, in Iberis[127].

Sterols are triterpenes which are based on the cyclopentane perhydrophenanthrene ring system. At one time, sterol were mainly considered to be animal substances (as sex hormones, bile acids, etc.) but in recent years, an increasing number of such compounds have been detected in plant tissues. Indeed, three so called phytosterols are probably

ubiquitous in occurrence in higher plants: sitosterol (formerly known as β-sitosterol), stigmasterol and campesterol. These common sterols occur both free and as simple glucosides. A less common plant sterol is α-spinasterol, an isomer of stigmasterol found in spinach, alfalfa and senega root. Certain sterols are confined to lower plants; one example is ergosterol, found in yeast and many fungi. Others occur mainly in lower plants but also appear occasionally in higher plants, e.g. fucosterol, the main steroid of many brown algae and also detected in the coconut.

Phytosterols are structurally distinct from animal sterols, so that recent discoveries of certain animal sterols in plant tissues are most intriguing. One of the most remarkable is of the animal estrogen, estrone, in date palm seed and pollen[128]. The occurrence of insect moulting hormones, the ecdysones, were discovered in plants[129] and have subsequently been found in a range of plant tissues.

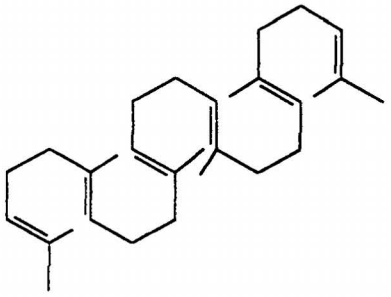
Saponins are glycosides of both triterpenes and sterols. They are surface active agents with soap-like properties and can be detected by their ability to cause foaming and to haemolyse blood cells. The search in plants for saponins has been stimulated by the need for readily accessible sources of sapogenins which can be converted in the laboratory to animal sterols of therapeutic importance (e.g.: cortisone, contraceptive estrogens etc.,). Saponins are also of economic interest because of their occasional

toxicity to cattle (e.g. saponins of alfaalfa) or their sweet taste (e.g. glycyrrhizin of liquorice root).

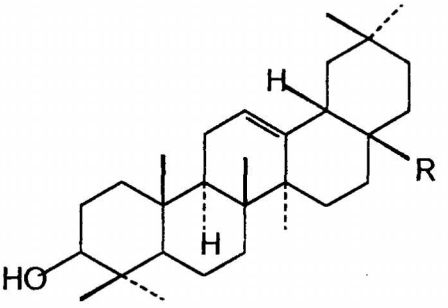
The last group of triterpenoids to be considered are the cardiac glycosides or cardenolides. A typical cardiac glycoside is oleandrin,the toxin from the leaves of oleander, Nerium oleander, Apocynaceae. An unusual structural feature of oleandrin and many other cardenolides is the presence of special sugar substituents, sugars indeed which are not found else where in the plant kingdom. Most cardiac glycosides are toxic and many have pharmacological activity, especially as their name implies on the heart. Special interest has been taken in the cardiac glycosides of Asclepias, because they are absorbed by Monarch butterflies feeding on these plants and are then used by the butterflies as a protection from predation by blue Jays[130]. The butterfly is unharmed by these toxins, which on other hand act as a violent emetic to the bird.

A number of cyclic triterpenoid compounds of the type taraxerol, friedelin and lanosterol have been reported to exhibit cyto toxic and anti - inflammatory activities.

Structures of some important Triterpenoids

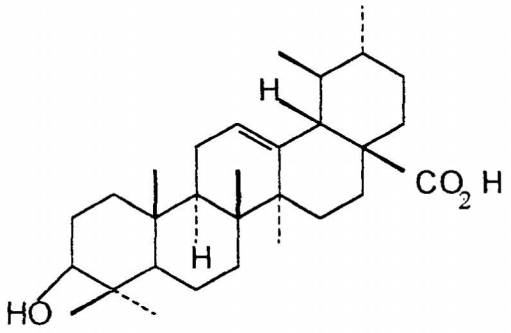


Squalene

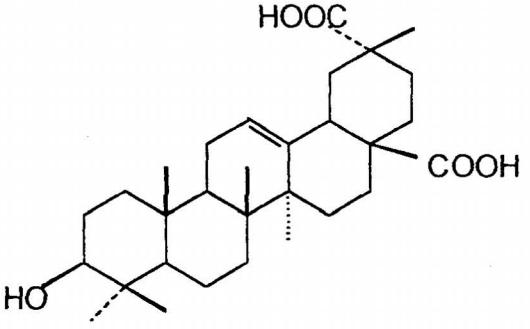


β - amyrin (R = Me)

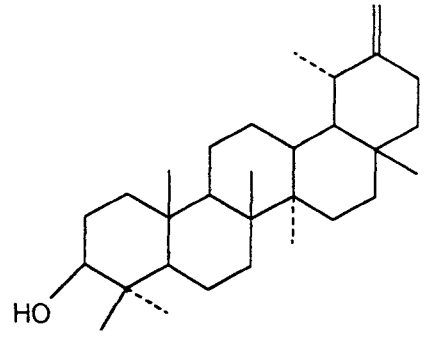
Oleanolic acid (R = CO_2H)



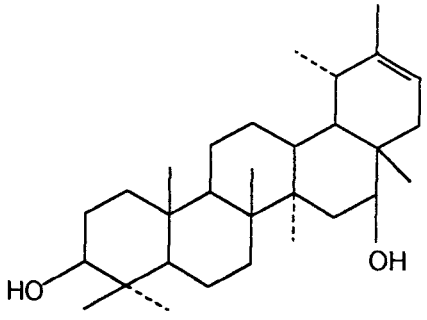
Ursolic acid



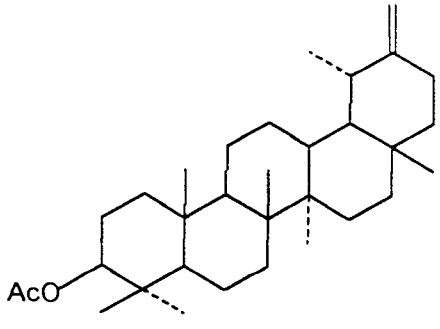
Serratagenic acid



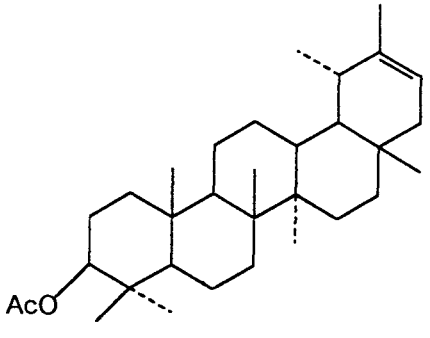
Taraxasterol



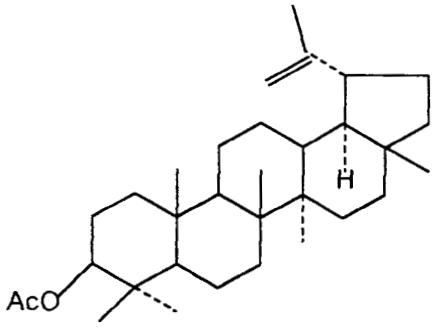
Faradiol



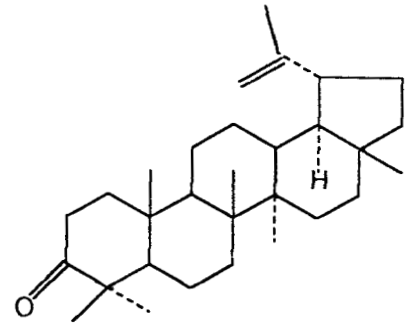
Taraxasteryl acetate



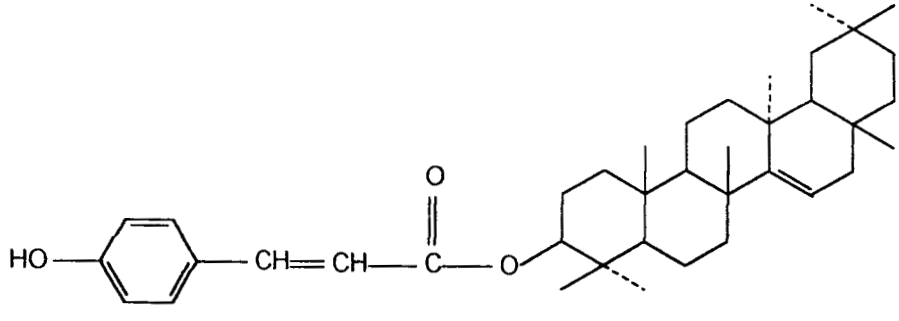
Ψ- taraxasteryl acetate



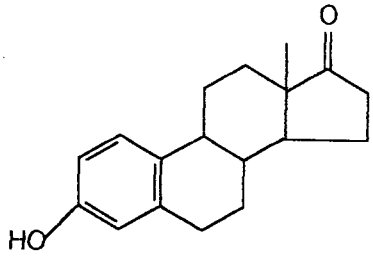
Lupenyl acetate



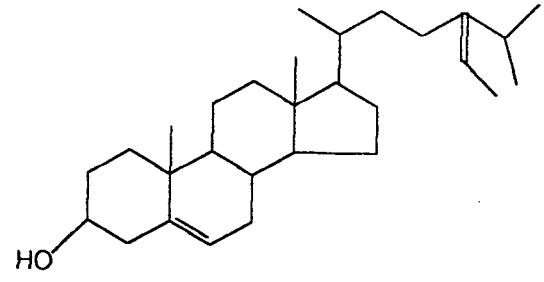
Lupenone



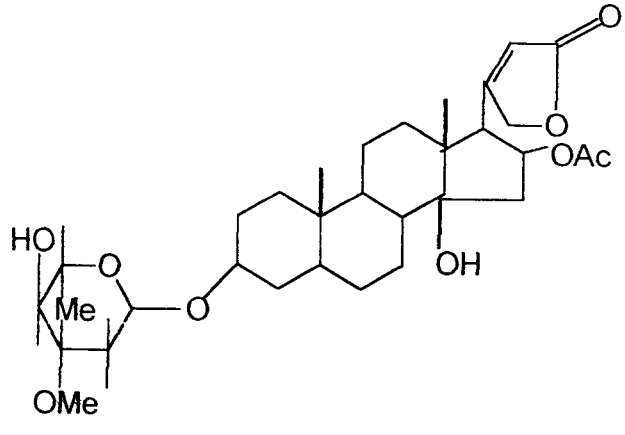
Taraxeryl - p - hydroxy Cinnamoate



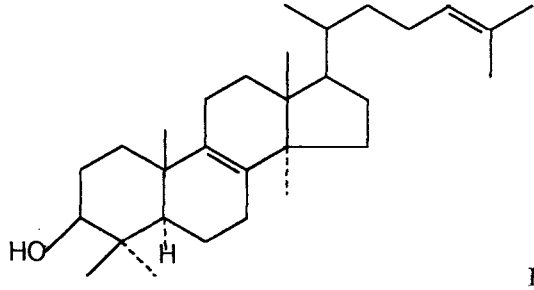
Estrone



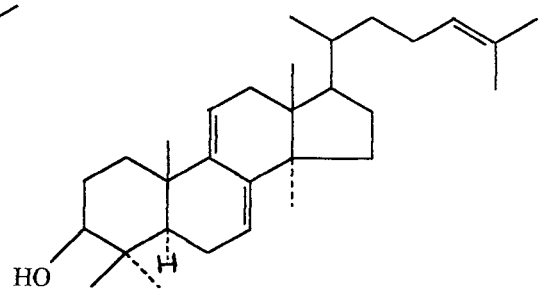
Fucosterol



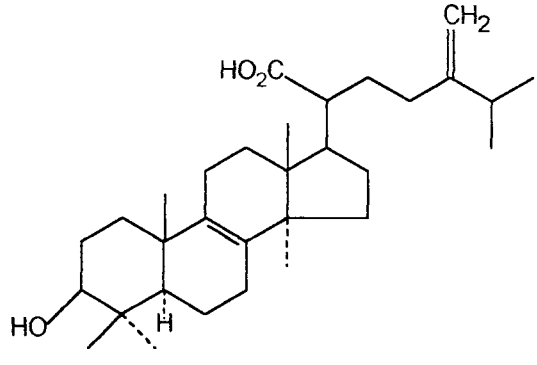
Oleandrin



Lanosterol



Agnosterol



Eburicoic acid

***Hibiscus schizopetalus* (Wall.) Hook. f.**

Phytochemical evaluation of Hibiscus schizopetalus

Jose E.A " Phytochemical evaluation of some plants used in folk medicine" Thesis. Department of Chemistry , University of Calicut, 2002

CHAPTER – II

PHYTOCHEMICAL EVALUATION OF *HIBISCUS SCHIZOPETALUS*

II.1. INTRODUCTION

Hibiscus schizopetalus (Wall.) Hook. f. belongs to the family Malvaceae. In Malayalam it is known as Kozhichemparuthi or Tookuchemparuthi. A variety of this plant has been found to be used in folk medicince[5].

H. Schizopetalus' are erect sub shrubs to four meter tall with many slender drooping branches. Stem is erect, long and slender. Leaves are ovate, elliptic, truncate at base, acute at apex and dentate; leaf blades 3.8 X 1.4 cm; Petioles 0.5 - 2 cm long, glabrous; stipules 2 - 3 cm long, subulate, glabrous. Flowers are axillary , solitary and pendent; pedicels 8 - 15cm long, slender, much exceeding the sub- tending leaves, articulated at about middle, glabrous. The calyx is long (1.5 - 20 cm), tubular, 5-toothed and glabrous. Corolla 5-6cm in diameter, red with white or

pink streakes; petals are strongly reflexed and deeply dissected, glabrous. Stamens are monodelphous and staminal column is long (8-10cm) and tubular. Lanthers monothecous; gynoecioum pentacarpellary, syncarpous, pentalocular with axile placentation; style simple, filiform; stigma pentafid. Fruit setting is not seen[131,132].

H. Schizopetalus' are found in almost all parts of India, Srilanka, South Africa and North America. They are now widely cultivated through out the tropics for its unusual and beautiful flowers.

II.2. PREVIOUS STUDIES REPORTED

A large number of Hibiscus species are reported to be medicinally important. Several species of Hibiscus genera have been found to be used as medicinal plants in folk medicine and so these form an ingredient in many of the Ayurvedic preparations. In traditional medicine, Hibiscus plants are extensively used for healing ulcers and for promoting the growth and colour of the hair. These plants are used in treating alopacia, burning sensation in the body, diabetes, menstrual disorder and piles[133]. The plants have been found to be effective in the treatment of arterial hypertension and anti fertility activities. Majority of these had been examined thoroughly for their active principles. Many of the compounds isolated from the plants of Hibiscus genera are found to be very active and are used in modern medicines. Taraxerol, Taraxeryl acetate and other

related compounds isolated from this genera have been used for anti-carcinogenic[134,135] and anti-inflammatory[136] activities. However, no literature is available regarding the secondary metabolites of the plant, *Hibiscus schizopetalus*. Search in literature has shown that this plant had not been phytochemically studied.

II.3. PRESENT WORK

In a pharmacological screening process to identify medicinal herbs with immunostimulant properties, a few species of Hibiscus genera of Malvaceae family are proved to be potential candidates for further phytochemical study. Some of them have been recognized as medicinal herbs in folk medicine and so these have been used as ingredients in many of the Ayurvedic preparations. For the present study, *Hibiscus schizopetalus* of Malvaceae family has been selected since, *Hibiscus schizopetalus* is one of the least phytochemically examined species of the genus, Hibiscus. So the present work is aimed to isolate the secondary metabolites from the leaves of the plant and analyze and identify them by chromatographic as well as spectroscopic methods.

II.4. RESULTS AND DISCUSSION

Shade dried and powdered leaves of *Hibiscus schizopetalus* was extracted repeatedly with petroleum ether in a Soxhlet extractor for 36 hrs. The combined extract was then concentrated under reduced pressure.

The extract was then adsorbed on 750g of silica gel and packed in a column. The column was eluted with petroleum ether, petroleum ether-ethyl acetate mixture of varying compositions and then with ethyl acetate alone. Different compounds isolated from the petroleum ether extract of leaves of *Hibiscus schizopetalus* were designated as **H-1, H-2, H-3, H-4** and **H-5.**

Different compounds obtained from the chromatographic purification is given in the **table 2.1.**

Table 2.1. Compounds isolated from the petroleum ether extract of *H. Schizopetalus'* leaves.

Compound	Eluant Composition	Melting point
H-1	Petroleum ether	41-43^{0}c
H-2	Petroleum ether - Ethyl acetate (9:1)	250-252^{0}c
H-3	Petroleum ether - Ethyl acetate (85:15)	245-247^{0}c
H-4	Petroleum ether - Ethyl acetate (4:1)	193-195^{0}c
H-5	Petroleum ether - Ethyl acetate (3:1)	260-262^{0}c

Thin layer chromatography can be used for the purification of compounds. The chromatograpic characterization of the compounds **H-1,H-2,H-3,H-4** and **H-5** is carried out using some selective colour forming spray reagents[139,140,141] such as Liebermann - Burchard, Vanillin-Sulphuric acid, Anisaldehyde-sulphuric acid, 20% sulphuric acid and alkaline potassium permanganate (Baeyer's reagent) reagents. With Liebermann - Burchard reagent triterpenoids are detected as red or pink spots and sterols and its esters are detected as green to blue spots. With vanillin-sulphuric acid reagent triterpenoids and steroids are detected as various coloured spots (red, yellow, blue or brown). With Anisaldelyde - Sulphuric acid reagent triterpenoids are detected as blue, red-violet, orange or red spots. With 20% aqueous Sulphuric acid, the terpenoids gave brown, pink, purple or yellow colour.

The results on TLC analysis of these different five compounds are given in **Table 2.2.**

Table 2.2.

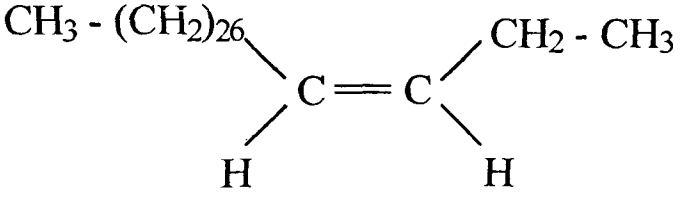
Thin layer chromatographic characterization of compounds isolated from the petroleum ether extract of *H.Schizopetalus'* leaves using spray reagents.

Compounds	Liebermann - Burchard ragent	Vanillin -sulphuric acid reagent	Anisaldehyde-sulphuric acid reagent	20% sulphuric acid	Baeyer's reagent	Class of compound
H-1	---	---	---	Dark brown	Decolourised	Olefin
H-2	Pink	Violet	Blue-violet	Pink	Decolourised	Triterpenoid
H-3	Pink	Blue	Blue-Violet	Pink	Decolourised	Triterpenoid
H-4	Blue - Green	Blue	Blue-Black	Pink	Decolourised	Steroidal Triterpenoid
H-5	Red	Blue-Black	Red - Violet	Brown	Decolourised	Triterpenoid

II.5. CHARACTERIZATION OF H-1(*CIS* - Hentriacont-3-ene)

The compound **H -1** was obtained as the first traction on elution of the column with petroleum ether. The compound was washed with cyclohexane several times and re-crystallized from n - hexane to yield a colourless waxy substance which melted at 41- 43^{0}C. This compound did not answer Liebermann-Burchand, Anisaldehyde-sulphuric acid

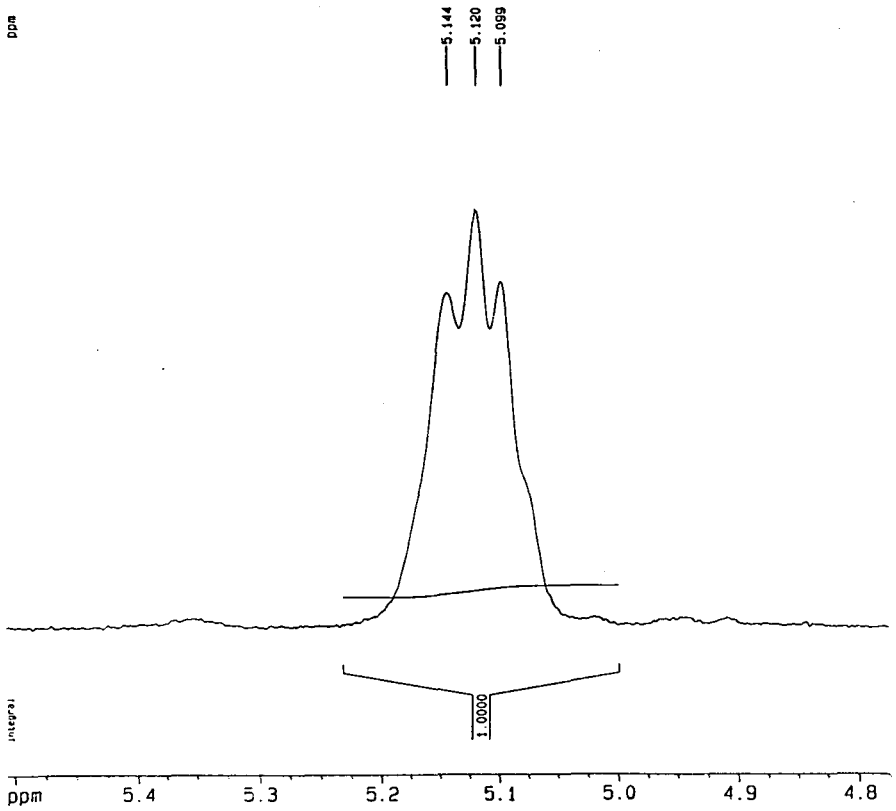
reagent and vanillin-sulphuric acid reagent colour reactions indicating that it is not terpenoid, triterpenoid or a steroid. It decolourised pink colour of alkaline potassium permanganate on spraying with Baeyer's reagent indicating that it is an unsaturated molecule. It gave a single spot on TLC analysis in various solvent systems. The detailed IR, ^{1}Hnmr, ^{13}Cnmr and mass spectral data investigations identified the compound as *cis*-Hentriacont-3-ene. The molecular formula was determined to be $C_{31}H_{62}$ by FABMS (M^{+}434.8308). The structure of the compound is identified as **H-1** based on the data obtained on the above spectroscopic studies.



H-1

IR spectrum gave medium absorption at 1629.7 cm^{-1} which was attributed to the presence of a C=C double bond. It also showed bands at 2920 cm^{-1} and 2850 cm^{-1} which was due to C – H stretching of – CH_2 (asym & sym), and bands at 1463.9 cm-1 and 1379 cm^{-1} showing C-H bending (aliphatic). Other bands seen in the spectrum at 3365 cm^{-1} and others are taken as due to the presence of moisture and can be ignored.

The ^{1}Hnmr & ^{13}Cnmr spectral data also agree very well with the suggested structure for the compound **H-1**. The ^{1}H signal at δ5.12 (2H, t,J=7.2 Hz) could be attributed to olefinic protons. The coupling constant value, J=7.2 Hz, shows that the olefinic protons are *cis* to each other and correspondingly the compound H-1 is a *cis*-olefin.

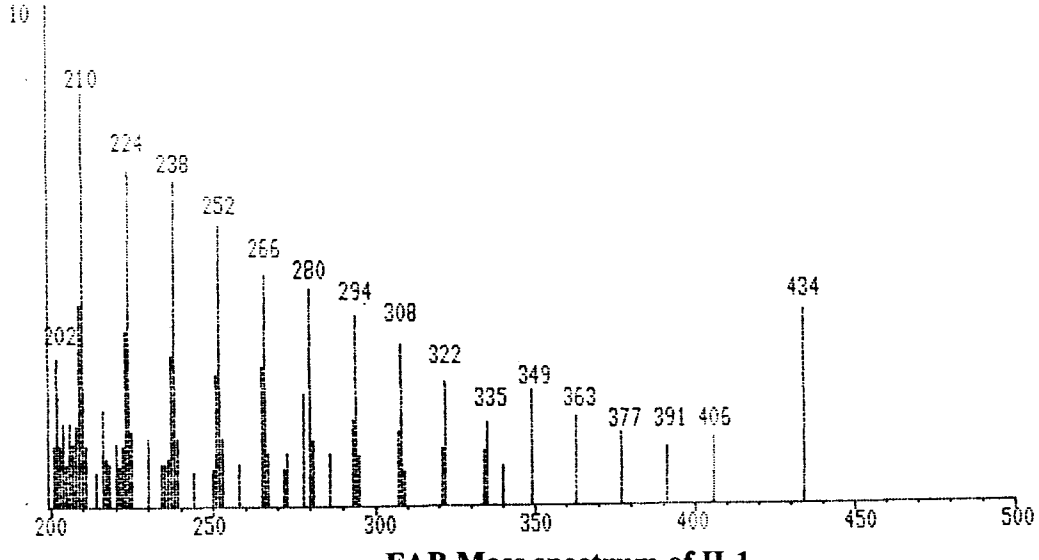


^{1}H-NMR spectrum of H-1(4.8-5.4 ppm)

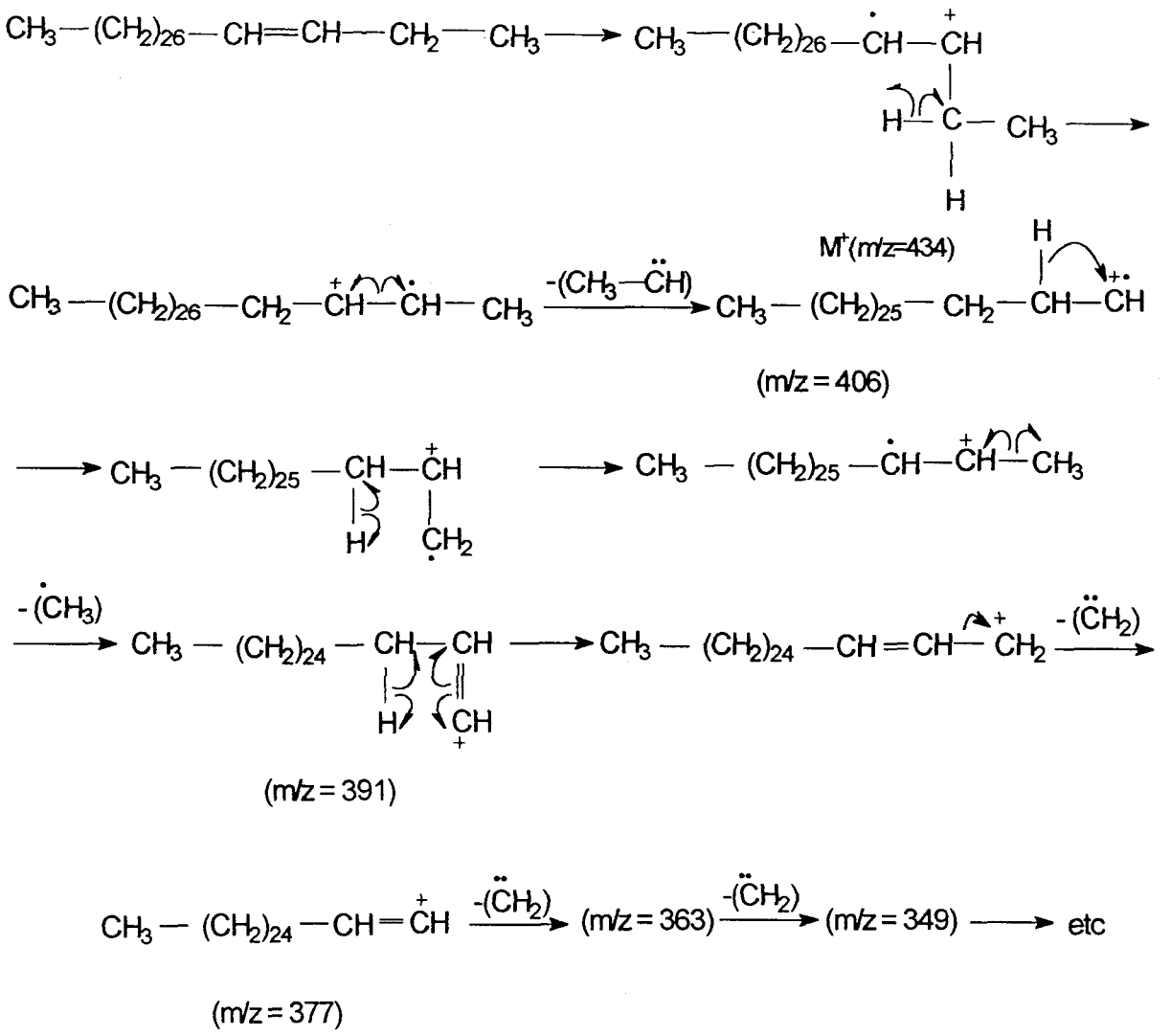
The ^{1}H signal at 2.009 ppm is ascribed to – CH_2 groups next to the double bond. The PMR chemical shifts at δ1.256, 1.602-1.681 were

assigned for all other CH_2 groups than those next to double bond. The other signals at δ_H 0.858 – 0.900 could be due to the two overlapping triplets of the two – CH_3 groups. The only disagreeing factor is that the integration of the olefinic protons seems to be only one. This may be due to the identical nature of the olefinic H atoms. This is supported by the ^{13}C and ^{13}C- DEPT spectral data. The signal at 123.5 ppm could be attributed to olefinic carbon atoms. The ^{13}C chemical shifts at δ13.3 to 40.2 could be assigned to the carbon atoms of – CH_3 and – CH_2 groups in the molecule.

In its mass spectrum the molecular ion (M^+) appeared at m/z 434. The other peaks at m/z values 406, 391, 377, 363, 349, 335.....etc are in agreement with the proposed formula **H-1**. The fragmentation pattern of the compound **H-1** can be depicted as given in **scheme –2.1.**



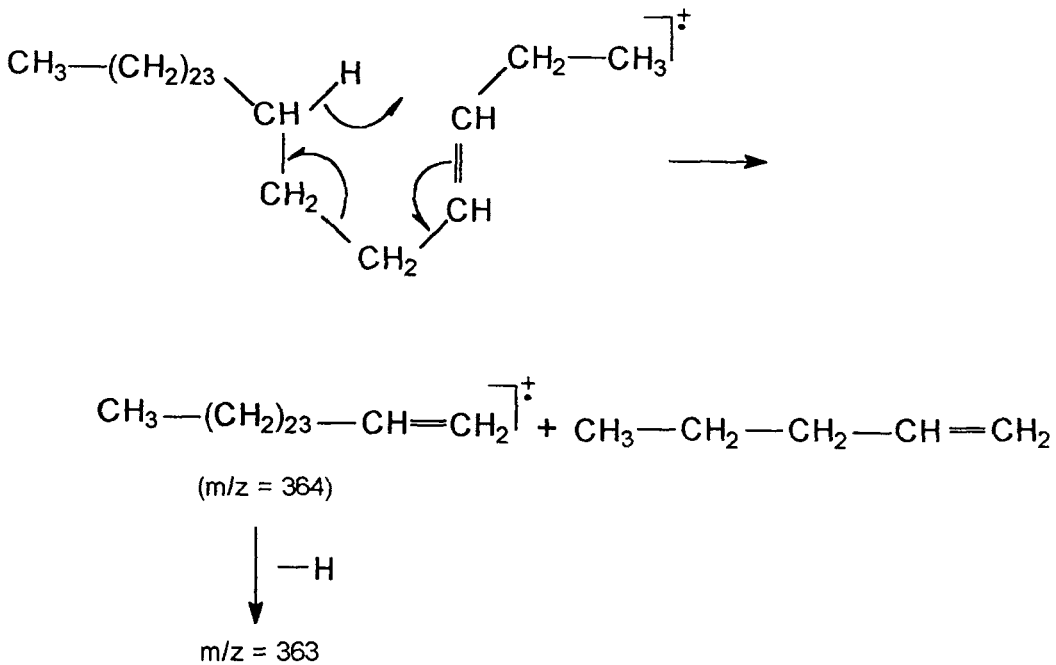
FAB Mass spectrum of H-1



Scheme – 2.1

The sequential loss of the mass units of 14 is characteristic of hydrocarbons (monoalknes). The double bond at 3 position could be confirmed by the (M^+-28) ion peak, the formation of which is shown in the scheme. The peak at m /e 406, i.e. M^+-28, is the second major peak after the molecular ion peak and this is in confirmation of the position of the double bond at 3.

The formation of the peak at m/z 363 can also be explained by a McLafferty rearrangement. This rearrangement again confirms that the position of the double bond is located at C-3. Also the m/z peaks at 71,57,44 etc present in the mass spectrum of the compound are characteristic peaks of mono alkenes. The rearrangement is as given in **scheme 2.2**.



Scheme - 2.2

Consideration of ^{1}Hnmr and ^{13}Cnmr data as well as IR and Mass spectral data allowed the complete structures of the compound to be assigned as c*is*-Hentriacont – 3 – ene.

The other compounds **H-2, H-3, H-4** and **H-5** isolated are discussed in following chapters separately.

ISOLATlON AND CHARACTERIZATION OF 22 - HYDROXYTARAXERYL ACETATE AND 22-HYDROXYTARAXERYL CIS- P - COUMARATE

Jose E.A " Phytochemical evaluation of some plants used in folk medicine" Thesis. Department of Chemistry , University of Calicut, 2002

CHAPTER III

ISOLATION AND CHARACTERIZATION OF 22 - HYDROXYTARAXERYL ACETATE AND 22-HYDROXYTARAXERYL - *CIS* - P - COUMARATE

The isolation and characterization of taraxeryl acetate from Taraxacum japonicum[134] (Compositae) and *Hibiscus rosa-sinensis*[137] (Malvaceae) plants and *cis* - and *trans* - p - hydroxycinnamoyl esters of Taraxerol from Bernardia laurentic[138] (Euphorbiaceae) had been reported earlier. These compounds are known for their potential anti-carcinogenic activities[134,135,137].

H. schizopetalus' leaf extract on column chromatography gave *cis*-Hentriacont -3 - ene as the first fraction, the characterization of which is described in the previous chapter. The column on further gradient elution gave two closely resolved fractions which are identified as triterpene esters. The spectral characteristics of the compounds are described herein. These two are identified as two new compounds which were not been reported earlier.

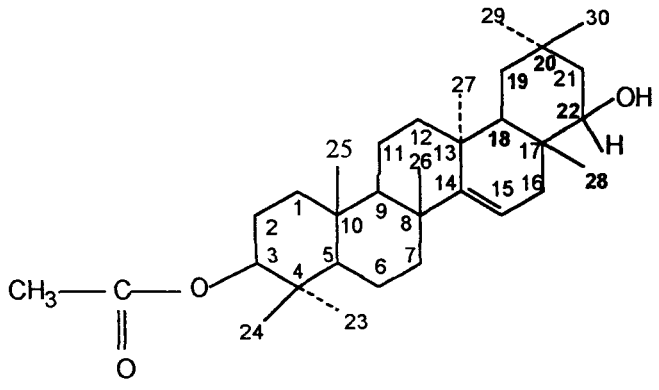
The compounds **H-2** and **H-3** isolated from the petroleum ether extract of leaves of *H. Schizopetalus* are identified with the help of IR, ^{1}Hnmr, ^{13}Cnmr and mass spectral data.

III.1. CHARACTERIZATION OF H-2 (22-Hydroxytaraxeryl acetate)

The Compound **H-2** was obtained on elution with petroleum ether and ethyl acetate mixture in the ratio 9:1. The compound thus obtained was purified by preparative TLC and recrystallized from benzene - ethyl acetate mixture (3:2). The white powder thus obtained melted at 250 - 252^{0}c. It gave a single spot on TLC analysis.

The compound **H-2** gave positive colour reactions with specific spray reagents indicating that it may be a triterpenoid. It gave a pink spot on spraying with Liebermann - Burchard reagent. With Vanillin - Sulphuric acid reagent the compound gave a violet colour. The examination of the compound with Anisaldehyde-sulphuric acid reagent gave blue-violet colour,and pink colour with 20% sulphuric acid reagent. It decolourised Baeyer's reagent indicating unsaturation in the molecule. A solution of the compound **H-2** when treated with Liebermann - Burchard reagent it gave green colouration, a characteristic colour reaction of triterpenoid compounds.

The detailed IR, ^{1}Hnmr, ^{13}Cnmr and Mass spectral data investigations identified the compound as 22- Hydroxytaraxer - 14 - en - 3β - ol - acetate (22-Hydroxytaraxeryl acetate) with the molecular formula $C_{32}H_{52}O_3$. The structure of the compound **H-2** can be depicted as follows:

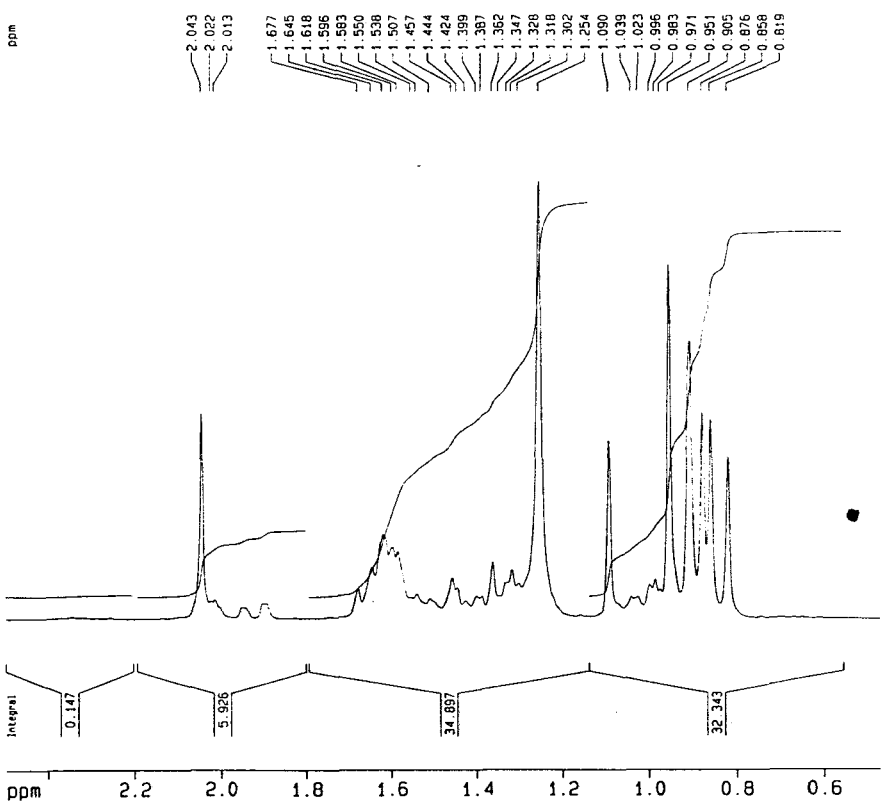


H-2

IR spectrum gave a strong absorption at 1724.2 cm^{-1} indicating the presence of an ester Carbonyl group and C - O - C stretch appears at 1251.7 cm^{-1}. A broad absorption between 3716 - 3265 cm^{-1} indicates the presence of an -OH group. It also showed bands at 2922 cm^{-1} and 2852.5cm^{-1} which was due to C - H stretching of $-CH_2$ (asym & sym) and bands at 1377.1 cm^{-1} and 1467.7 cm^{-1} were due to C - H bending. Other band seen in the spectrum at 1649 cm^{-1} was attributed to the presence of C = C double bond stretching.

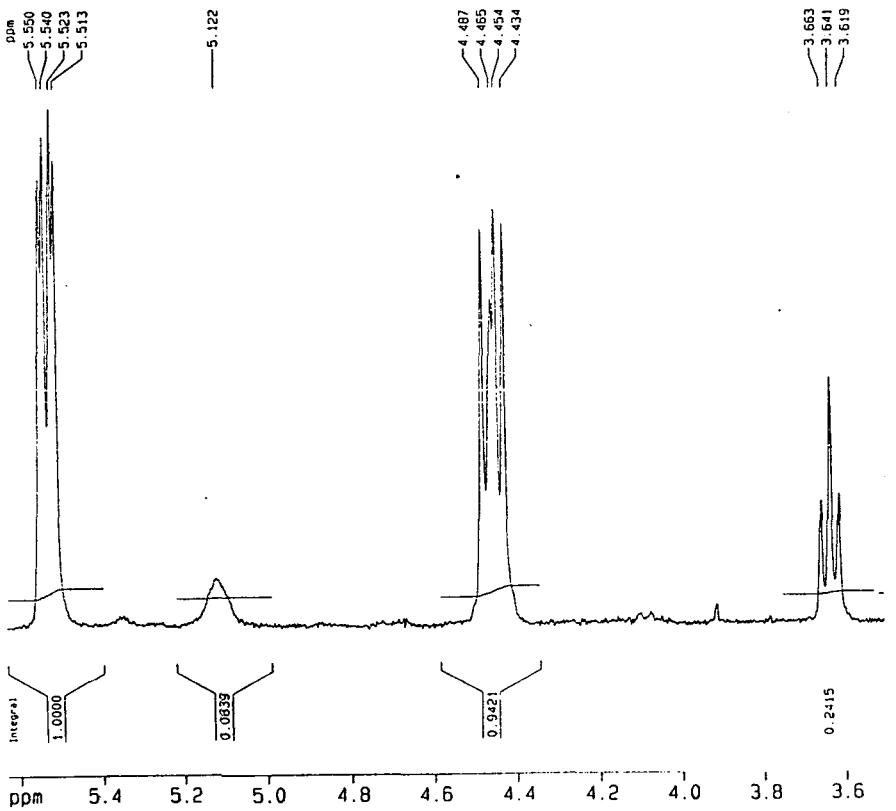
Based on the colour reactions with various spray reagents, the compound **H-2** was proved to be pentacyclic tripterpene. Also the ^{1}H - and^{13}C-NMR spectra exhibited a pentacyclic triterpenoid pattern. The^{1}H - and ^{13}C - NMR spectral data also agree very well with those reported for taraxeryl acetate[134] with some additional features. In the PMR spectrum it gave signals at δ0.819 - δ1.090 (all singlets) indicating the presence of eight tertiary methyl groups. The ^{13}C spectra and DEPT[142] edited spectra also agrees with this observations. The DEPT - 90^{0} and DEPT - 135^{0} spectra accounts the presence of six CH (methine)carbons, nine-CH_2 carbons and eight -CH_3 carbons. But there are only twelve positive peaks in DEPT-135^{0} spectra. Actually there should have been a total of fourteen positive peaks accounting for six CH and eight CH_3 carbons. We have assigned the same value for C-3 and C-22 the oxygen bearing carbons (81.1ppm) and C-28 and C-30 carbons (29.9ppm). Hence a total of 14 peaks could be accounted for. In almost all of the published ^{13}Cnmr data for pentacyclic triterpenes having methyl groups on similar angular and gem dimethyl positions same value of assignments are given.[138,143,144]. Also in the DEPT - 135^{0} edited spectra two negative peaks at δ35.16 and δ29.73 can be taken as false peaks. In the ^{1}H spectra, the tertiary methyl groups appeared as singlets at δ0.819, δ0.858, δ0.876 integrating for three protons each indicated the presence of three methyl protons. The singlet

signals at δ0.905 and δ0.951 are due to four methyl protons which are attributed to the gem dimethyl groups at C-4 and C-20. A singlet signal at δ1.090 is due to the methyl protons of C - 26 which was attached to the Carbon - 8. The values assigned are given in **Table 3.1**. The additional - OH group in the taraxerol ring skeleton than that reported for taraxeryl acetate can be proved by IR absorption band at 3907 - 3265 cm^{-1} and from ^{1}H- and ^{13}C-NMR signals. The PMR signal and δ5.122 (broad) indicates the presence of -OH group. The ^{13}C- and ^{1}H-NMR spectral data showed that there were two CH groups with ^{13}C and ^{1}H chemical shifts characteristic of an attached oxygen functionality.



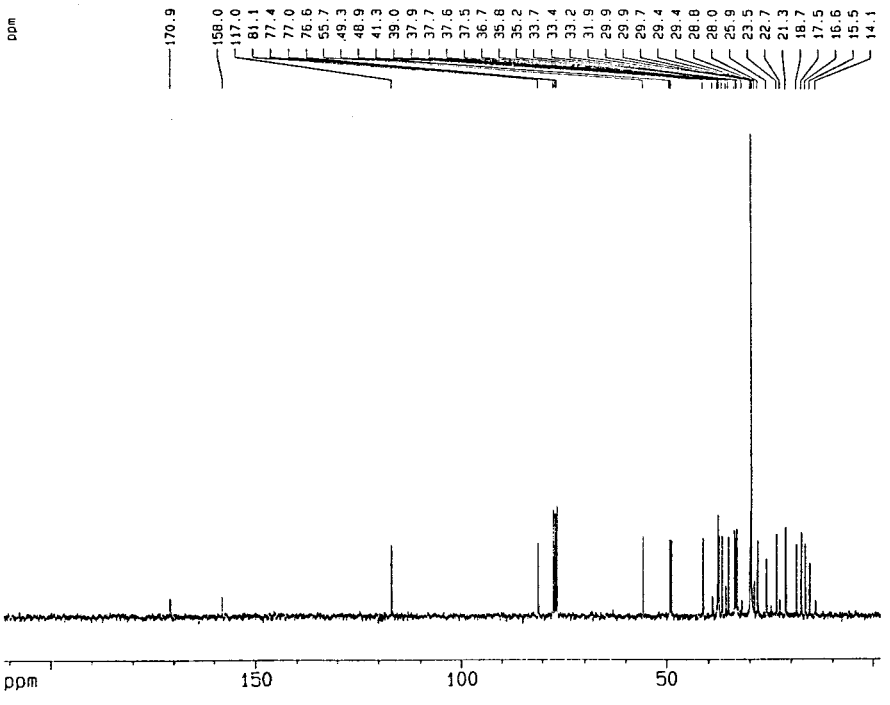
^{1}H-NMR spectrum of H-2 (0.6 - 2.4 ppm)

The DEPT - 90^0 edited spectra also ascertains this observation. The ^{13}C signal at 81.1ppm can be attributed for both C - 3 and C - 22. The ^1Hnmr signal at δ4.459 which is obtained as a double doublet (J = 6.6, 9.6Hz) can be attributed to the H at position C - 3. The double doublet (J=6.6, 9.6 Hz) formation can be justified because it interacts with hydrogen on C - 2 and C - 5. ^1H-^1H COSY spectral studies by Hisashi Matsuda et al on other pentacyclic tripterpenes supports this observations[146]. The signal at δ3.641 (t, J = 6.6 Hz) which is a 1H triplet can be attributed to the H at position C - 22. Incidentally the OH group has been placed at C - 22 and not on other positions like C - 21, which is also possible by the spectral data, on phytochemical considerations[147,148] .



^1H-NMR spectrum of H-2 (3.6 - 5.6 ppm)

Also ^{1}Hnmr signal at 5.532 ppm, which is obtained as a double doublet (J = 3, 8.1 Hz) was assigned to olefinic H at C - 15 as found in many triterpenes[145]. The double doublet (J = 3, 8.1 Hz) formation can be justified because it interacts with bydrogen on C - 16 and C - 18 as is evident from co-relational spectrosopic study of pentacyclic triterpenes. The ^{13}C signals at 158 ppm and 117 ppm can be attributed to C - 14 and C - 15 respectively. Also the ^{1}Hnmr signal at δ2.043 (s) can be attributed to acetyl methyl protons. The ^{13}C signal at 170.9 ppm was assigned to acetyl carbon and 21.3 ppm was attributed to acetyl methyl carbon. The ^{13}C signals at 76.6 - 77.4 ppm are due to the solvent $CDCl_3$ carbon.



^{13}C-NMR spectrum of H-2

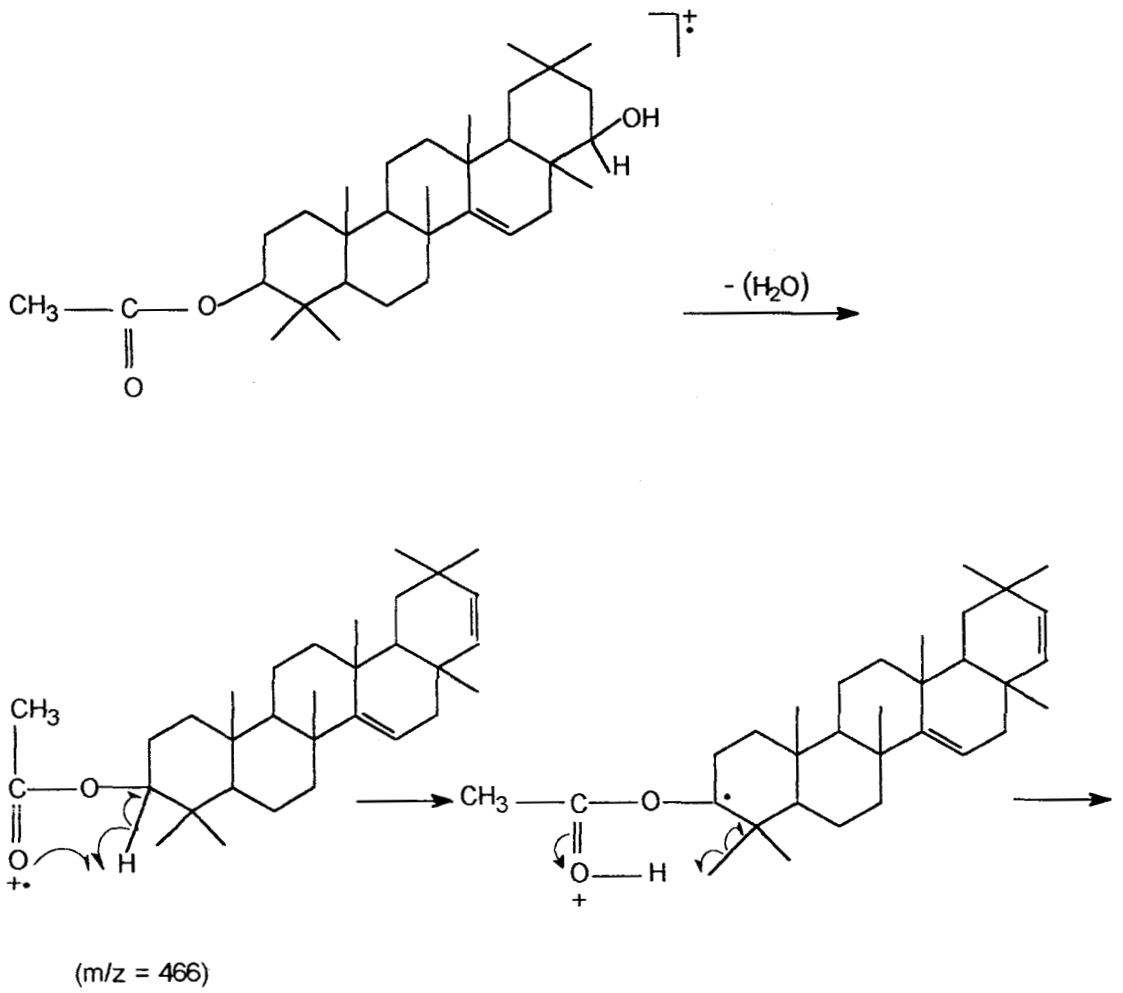
Table 3.1.
^{13}C- and ^{1}H- NMR assignments (recorded in $CDCl_3$) for 22 - hydroxy taraxeryl acetate (H-2)

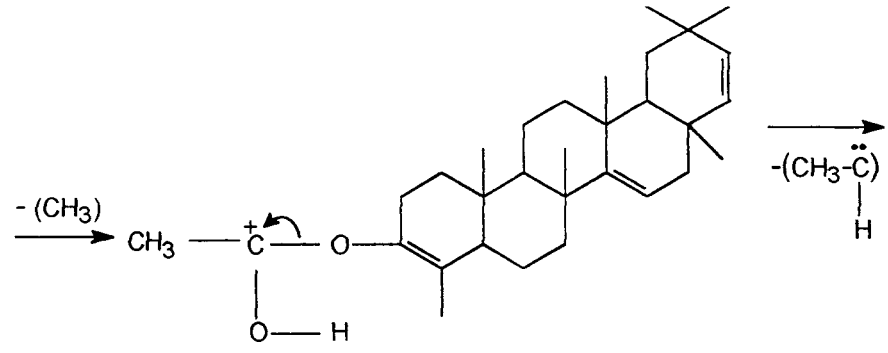
Carbon[1]	DEPT	δc	δH	Carbon[1]	DEPT	δc	δH
1.	CH_2	37.5	1.254	**18.**	CH	48.9	1.302
2.	CH_2	23.5	1.645	**19.**	CH_2	36.7	1.347
3.	CH	81.1	4.459(dd)	**20.**	C	28.8	---
4.	C	37.9	---	**21.**	CH_2	33.2	1.362
5.	CH	55.7	1.254	**22.**	CH	81.1	3.641(t)
6.	CH_2	18.7	1.387	**23.**	CH_3	28.0	0.905 (s)
7.	CH_2	41.3	1.44	**24.**	CH_3	16.6	0.951 (s)
8.	C	39.0	---	**25.**	CH_3	15.5	0.876(s)
9.	CH	49.3	1.318	**26.**	CH_3	25.9	1.090 (s)
10.	C	37.9	---	**27.**	CH_3	21.3	0.858(s)
11.	CH_2	17.5	1.424	**28.**	CH_3	29.9	0.819 (s)
12.	CH_2	33.7	1.399	**29.**	CH_3	33.4	0.951(s)
13.	C	37.6	---	**30.**	CH_3	29.9	0.905 (s)
14.	C	158	---	-CO-CH_3	CH_3	21.3	2.043 (s)
15.	CH	117	5.532(dd)	-CO_2 -	C	170.9	---
16.	CH_2	37.7	1.677	- O - H	---	---	**5.122 (br)**
17.	C	35.8	---				

1. Numbering as shown in Structure of **H-2**

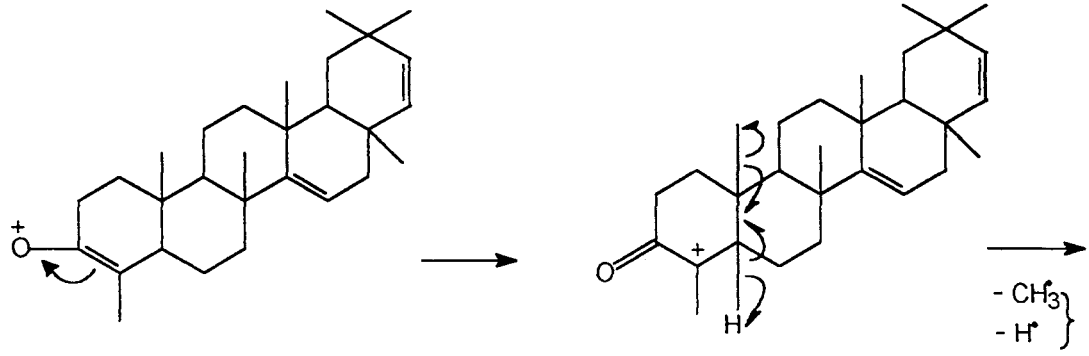
In the mass spectrum molecular ion signal at m/z 484 is not seen. This is a common occurrence in the case of steroidal and pentacyclic triterpenoids. Usually the ion with highest m/z value will be the (M^+ -18) peak. This is obtained in the case of this compound also. The signal at m/z 466 is explained as (484 - H_2O)[135]. This ion would have been formed

by dehydration reaction involving the hydroxyl at C-22 and a hydrogen from C-21. The other peaks at m/z values 451, 407, 391, 344, 343 and the base peak at m/z 203 are quite consistent with the proposed formula **H-2**. Possible structures for the selected ions[147,149,150] in the mass spectrum of the compound H_2 can be depicted as given in **scheme: 3.1.**

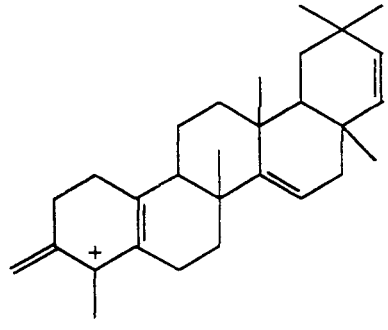




(m/z = 451)



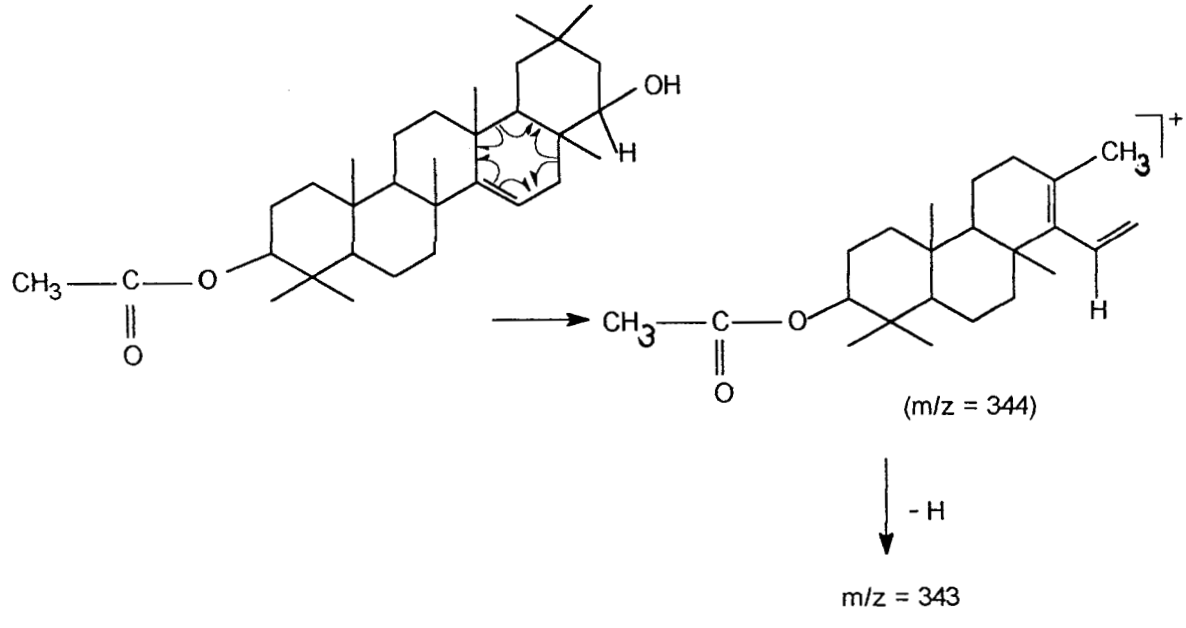
(m/z = 407)



(m/z=391)

Scheme:3.1

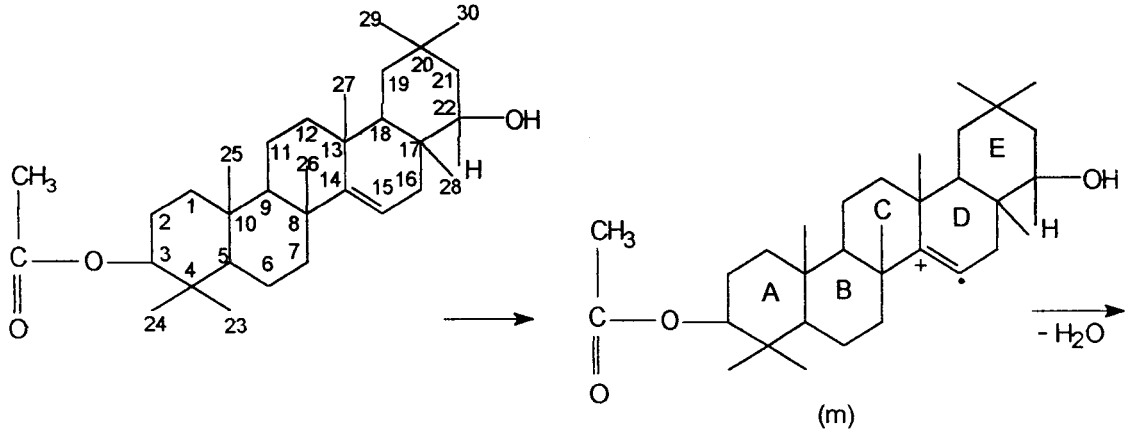
The most characteristic fragmentation of all compounds of the class pentacyclic triterpenoids can be described best by a retro-Diels-Alder reaction. This type of fragmentation has been described as an energetically very favoured process and has actually been observed in many cases.[149,150,151] In this case, retro- Diels - Alder fragmentation yields the ion at m/z 344, which loses a H atom to give the peak at m/z 343. The retro- Diels - Alder reaction is an important fragmentation pathway for cyclic alkene systems which is helpful in establishing the position of a double bond. The fragmentation is given in **scheme: 3.2.**

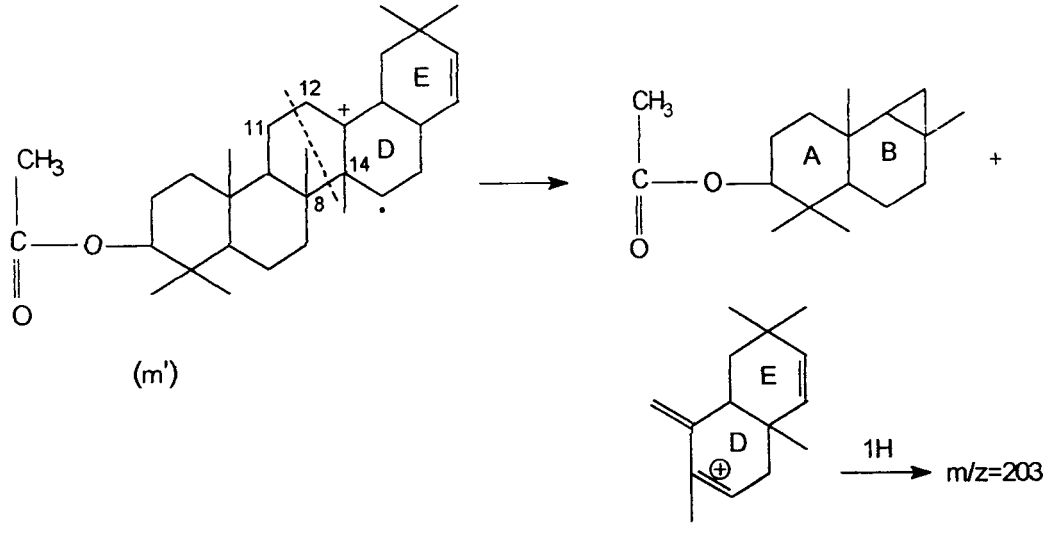


Scheme: 3.2

This confirms the lower part of the molecule and the position of the double bond at C_{14} - C_{15}.

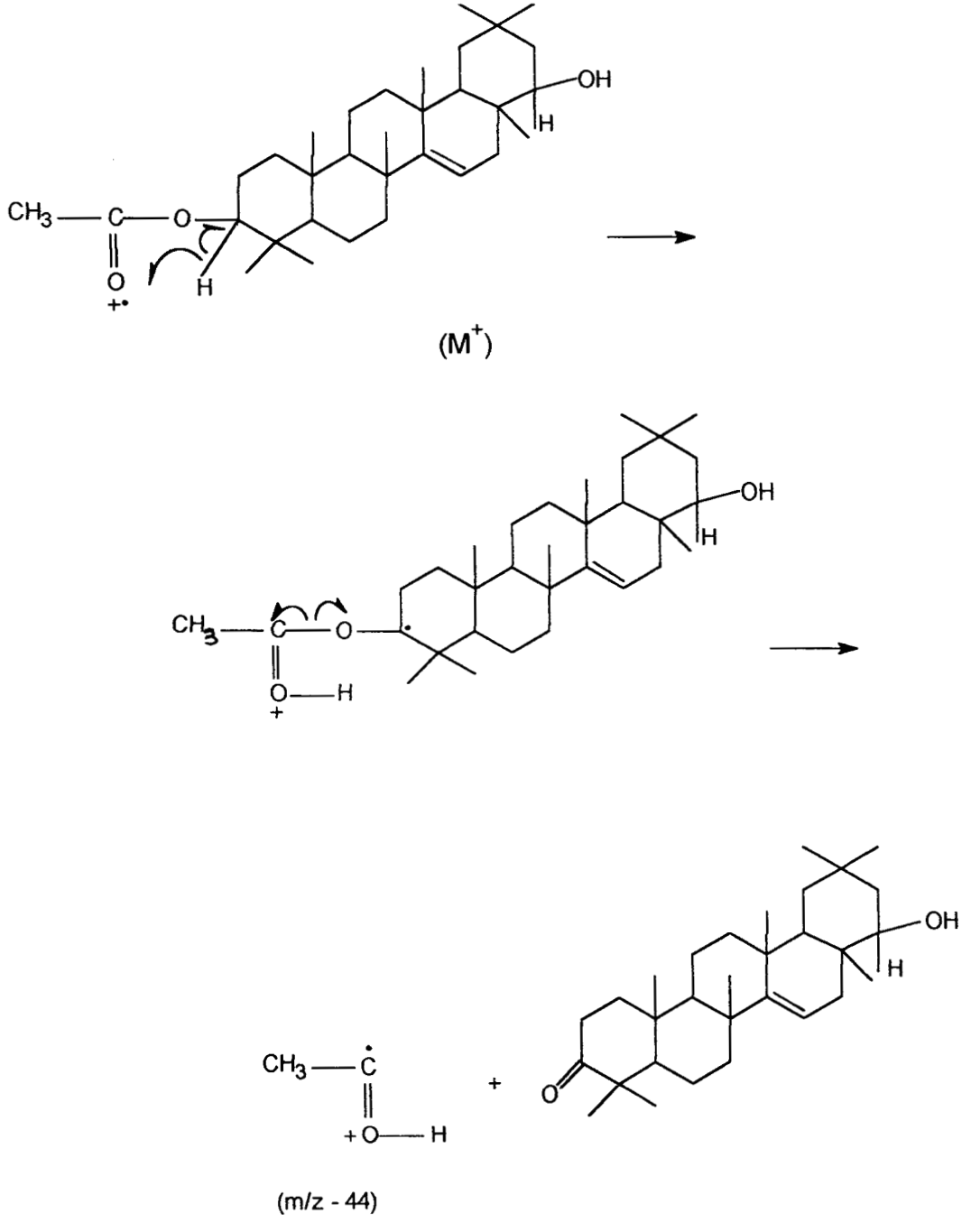
The formation of the base peak at m/z = 203 can be envisaged to be formed by the following mechanism, as suggested by H.Budzikiewicz et al,[149,150] by assuming that in the molecular ion the missing electron is preferentially removed from the carbon - carbon double bond (m); then migration of C-13 methyl group and the loss of water molecule is taking place yielding the radical ion (m'). Fission of the 11-12 and 8-14 bonds now gives the stable dieine. The pattern is given in **scheme: 3.3.**





Scheme:3.3

The other prominent peak at m/z=44 can be explained as due to the acetyl moiety of the compound **H-2**. The formation of this ion can be depicted as given in **scheme:3.4**.



Scheme: 3.4

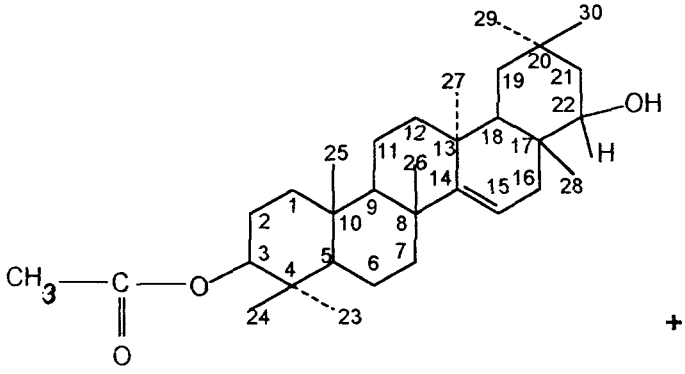
Consideration of ^{1}Hnmr and ^{13}Cnmr data as well as IR and Mass spectral data allowed the complete structure of the compound **H-2** to be assigned as 22 - Hydroxytaraxer - 14 - en - 3β- ol - acetate (22- Hydroxytaraxeryl acetate) with molecular formula $C_{32}H_{52}O_3$.

III. 2. CHARACTERIZATION OF H-3

The compound **H-3** was obtained on elution with petroleum ether and ethyl acetate combination in the ratio 85:15 as the third fraction. The solvent was removed by evaporation and the compound on recrystallization from benzene - ethyl acetate (1:1), it was obtained as white powder. It melted at 245 - 247^{0}C.

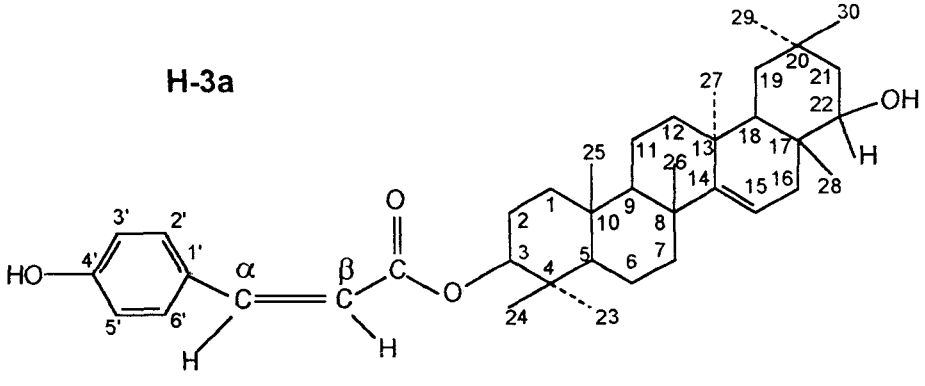
This compound, **H-3** gave positive colour reactions with specific spray reagents indicating that it may be a triterpenoid. It gave a pink spot on spraying with Liebermann - Burchard reagent. With Vanillin - Sulphuric acid reagent the compound gave a blue colour. The examination of the compound with Anisaldehyde - Sulphuric acid reagent it gave blue - violet colour. With 20% Sulphuric acid reagent the compound gave pink colour. It decolourised pink colour of alkaline potassium permanganate on spraying with Baeyer's reagent indicating unsaturation in the molecule. A solution of the compound **H-3** when treated with Liebermann - Burchard reagent a greenish yellow

colour is obtained. Also it gave a light blue colouration with neutral ferric chloride indicating a phenolic hydroxyl group in the molecule. Though a single spot is obtained on TLC analysis, the elongated nature of the spot may be due to a mixture of compounds having comparable R_f values. The detailed IR, PMR, ^{13}Cnmr and Mass spectral data investigations identified the **H-3** as an intimate mixture of 22 - Hydroxytaraxeryl acetate (22-Hydroxytaraxer-14-en-3β-ol-acetate), **H-3a,** with the molecular formula $C_{32}H_{52}O_3$ and 22 - Hydroxytaraxeryl *-cis* - p - coumarate (22-Hydroxytaraxer-14-en-3β-ol-*cis*-p-coumarate),**H-3b,** with the molecular formula C_{39} H_{56} O_4. The compounds were assigned the following structures.



H-3a

+



H-3b

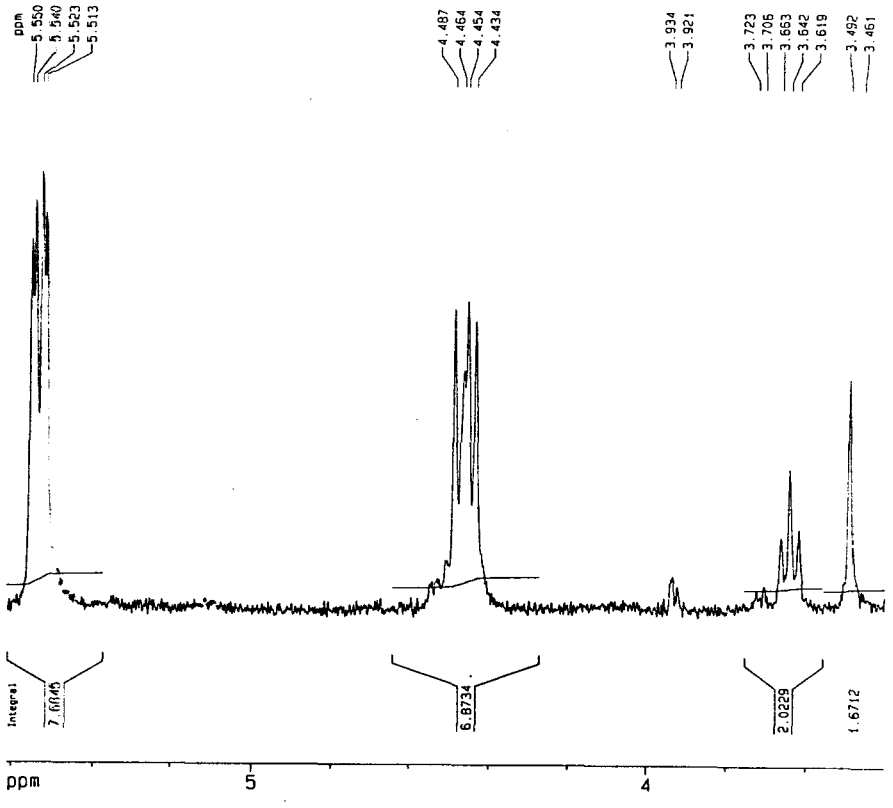
The compound on IR spectral analysis gave a strong absorption at 1726 cm^{-1} and a weak absorption as a shoulder at 1697 cm^{-1}. The small absorption peak in the IR spectrum at 1697 cm^{-1} is attributed to the α - β unsaturated ester carbonyl group in compound **H-3b** and the strong absorption peak at 1726 cm^{-1} is due to the acetoxyl carbonyl group in Compound **H-3a**. A broad IR absorption in the 3700 - 3300 cm^{-1} region indicate the presence of hydroxyl (both phenolic and alcoholic) group in the compound. It also showed bands at 2929.7 cm^{-1} and 2856.4 cm^{-1} which was due to C - H stretching of CH_2 (asym & sym) and bands at 1375.2 cm^{-1} and 1461.7 cm^{-1} were due to C - H bending. The absorption at 1251.7cm^{-1} was due to C - O - C stretching. The other peak seen in the spectrum at 1631.7cm^{-1} was attributed to the presence of C = C double bond stretching.

The ^{13}C- and ^{1}H-NMR spectral data are similar to those reported for *cis* - p - hydroxy cinnamoyl ester[138] of Taraxerol with some additional signals. The values are given in **table 3.2**. A signal in the IR spectrum of the compound which is a broad absorption at 3361.7cm^{-1} is indicative of of an secondary alcoholic group in Taraxerol ring skeleton than that is reported for c*is* - p - hydroxy cinnamoyl ester of taraxerol. The ^{1}H- and ^{13}C- NMR spectral data exhibited a pentacyclic triterpenoid pattern. In the PMR spectrum it gave signals at 0.819 - δ1.090 (singlets) showing

the presence of eight tertiary methyl groups. The ^{13}C spectra and DEPT edited spectra also agrees with this observations. The DEPT - 90^0 and DEPT -135^0 spectra shows the presence of five CH carbons and nine CH_2 carbons. In the DEPT-135^0 edited spectra two negative peaks at 35.13 ppm and 29.73 ppm are seemed to be false peaks. Also we have assigned the same value for C-3 and C-22, the oxygen bearing carbon (81.1 ppm) and C-28 and C30 (29.9ppm). Hence all the peaks in the DEPT-135^0 spectra for the CH & CH_3 (as positive peaks) and CH_2 (as negative peaks) could be accounted. In the ^1H spectra, the tertiary methyl groups appeared as singlets at δ0.819, δ0.858, δ0.876 and indicated the presence of three methyl groups. The singlet signals at δ 0.905 and δ 0.951 are due to 4 methyl groups which are attributed to the gem dimethyl groups at C - 4 and C - 20. A singlet signal at δ1.090 is due to the methyl protons of C - 26 which was attached to the C - 8. The ^{13}C- and ^1H-NMR spectral data showed that there were two CH groups with ^{13}C and ^1H chemical shifts characteristic of an attached oxygen functionality. The DEPT - 90^0 edited spectra also ascertains this observation. The ^{13}C signal at 81.1ppm can be attributed for both C - 3 and C - 22. The ^1Hnmr signal at δ4.459 which is obtained as a double doublet (J = 6.6, 9.6 Hz) can be attributed to the H at position C - 3. The double doublet (J = 6.6, 9.6 Hz) formation can be justified because it interacts with hydrogen on C - 2 and C - 5. The PMR

signal at δ3.642 (t, J = 6.6 Hz) which appeared as triplet can be assigned to the H at position C - 22. Incidentally the -OH group has been placed at C - 22 and not on other positions like C - 21, which is also possible by the spectral data, on biosynthetic considerations[147,148]. The ^{1}Hnmr spectral data showed that there is one double bond also in the skeleton with a single olefinic proton. This proton signal is obtained at δ 5.532 (dd, J = 3 and 8.1Hz) and was assigned for olefinic proton at C - 15 as found in many triterpenes. The double doublet (J = 3 and 8.1Hz) formation can be justified because it interacts with hydrogen on C - 16 and C - 18 as is evident from correlation spectroscopic study of pentacyclic triterpenes. The ^{13}C signals at 158 ppm and 117 ppm can be attributed to C -14 and C -15 respectively. Also the ^{1}Hnmr signal at δ 2.043 (s) can be attributed to acetylmethyl protons. The ^{13}C signal at 171.1ppm was assigned to - CO_2 - carbon.

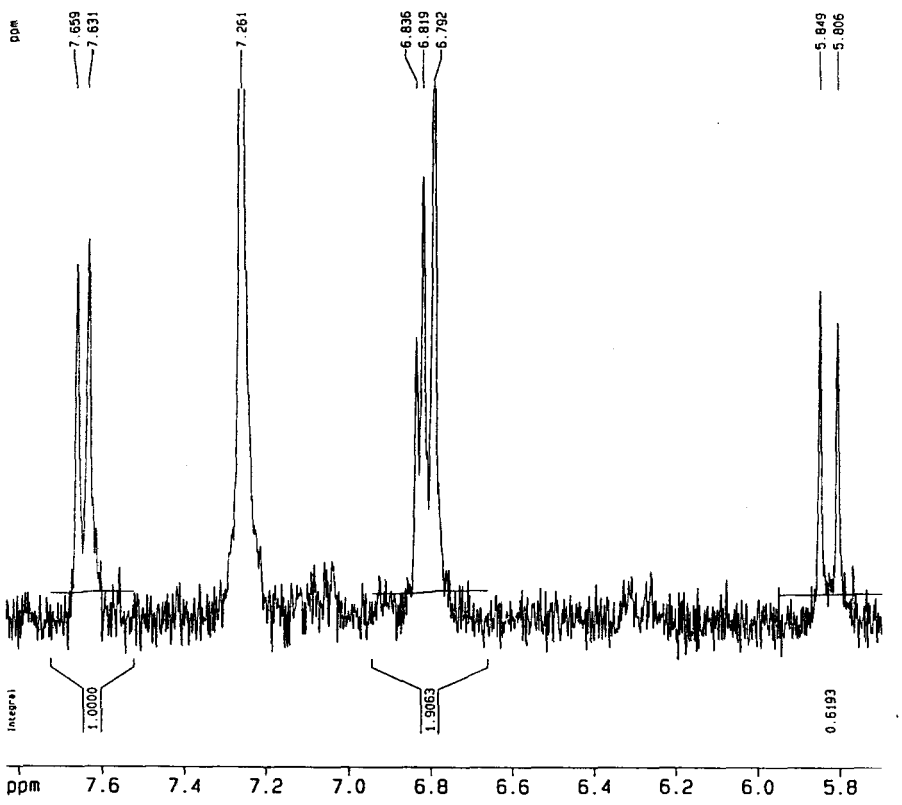
Inspection of the aromatic and olefinic region of the ^{1}Hnmr and ^{13}Cnmr spectral data of the compound (see table 3.2) suggested that it contains a para disubstituted benzene ring and a *cis* - disubstituted olefinic group. The well resolved PMR signals at 7.645 (2H, d, J = 8.4Hz) and 6.805 (2H, d, J = 8.1Hz) could be ascribed to 2',6' and 3',5' protons of the phenyl ring in the ester part respectively. The ^{13}Cnmr signal at 132.4ppm and 115 ppm can be attributed to 2',6' and 3',5' carbon atoms of



^{1}H-NMR spectrum of H-3 (3.4 - 5.6 ppm)

the phenyl ring in the ester part. The ^{1}Hnmr signals at 6.836 (d, J = 13.2 Hz) which is formed as a doublet integrated for 1H with coupling constant, J = 13.2 Hz and δ 5.83 (d, J = 12.9 Hz) which is formed as a doublet integrated for 1H with coupling constant, J = 12.9 Hz could be attributed to α- and β- olefinic H atoms of the cinnamoyl ester functionality. From the values of PMR signals and coupling constants, it can be assumed that the olefin is *cis* substituted[138]. In addition to this ^{13}Cnmr chemical shifts also confirms this observations. Also ^{1}Hnmr spectrum showed a signal at 2.043ppm which could be attributed to

acetyl methyl protons and 21.3ppm was assigned to acetyl methyl carbon. Now it can be stated that all the spectral data for the compound agrees with the proposed structure. Moreover, these data are compared with available published ^{13}C and ^{1}Hnmr data for the cinnamoyl and acetyl esters of tarxerol and other pentacyclic triterpenes[138,143,144,146].



^{1}H-NMR spectrum of H-3 (5.8 - 7.8 ppm)

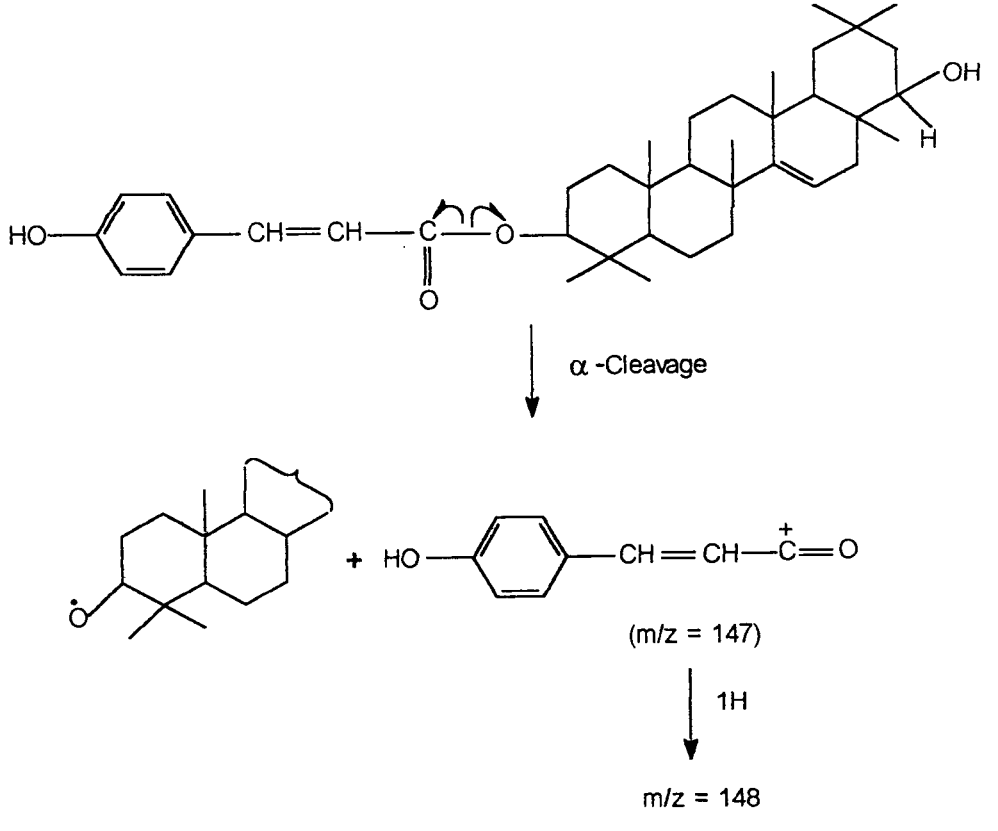
The ^{1}H- and ^{13}C- NMR assignments for the compound [H-3] are given in **table 3.2**

Table 3.2
^{13}C- and ^{1}H-NMR assignments (recorded in $CDCl_3$) for H-3

Carbon[1]	DEPT	δc	δH	Carbon[1]	DEPT	δc	δH
1.	CH_2	37.4	1.254	21.	CH_2	33.1	1.362
2.	CH_2	23.5	1.645	22.	CH	81.1	3.642 (t)
3.	CH	81.1	4.459(dd)	23.	CH_3	28	0.905 (s)
4.	C	37.9	---	24.	CH_3	16.6	0.951 (s)
5.	CH	55.7	1.254	25.	CH_3	15.5	0.876 (s)
6.	CH_2	18.7	1.387	26.	CH_3	25.95	1.090 (s)
7.	CH_2	41.3	1.44	27.	CH_3	21.3	0.858 (s)
8.	C	39	---	28.	CH_3	29.9	0.819 (s)
9.	CH	49.2	1.318	29.	CH_3	33.4	0.951 (s)
10.	C	37.9	---	30.	CH_3	30	0.905 (s)
11.	CH_2	17.5	1.424	1'	C	127	---
12.	CH_2	33.7	1.399	2',6'	CH	132.4	7.645 (d)
13.	C	37.6	---	3',5'	CH	115	6.805 (d)
14.	C	158	---	4'	C	157	----
15.	CH	117	5.532(dd)	α	CH	143	6.836 (d)
16.	CH_2	37.7	1.677	β	CH	117	5.83 (d)
17.	C	35.8	---	-CO-**CH3**	CH_3	21.3	2.043 (s)
18.	CH	48.8	1.302	$-CO_2-$	C	171.1	---
19.	CH_2	36.7	1.347	Phenolic OH		---	3.934
20.	C	28.8	---	Alcoholic OH		---	3.492

[1] Numbering as shown in structures for **H-3a** and **H-3b**

In the mass spectrum molecular ion signals for compounds **H-3a** and **H-3b** are not observed. The m/z signals at 466,451, 407. 391....are same as that expected for the compound **H-3a** (see **scheme 3.1**). The m/z peaks at 344, 343 and 203 are quite consistent with the proposed structures for the compounds **H-3a** and **H-3b** (see **scheme 3.2** and **3.3**). This confirms the lower part of the molecule of the compounds **H-3a** and **H-3b** and the position of the double bond at C^{14} - C^{15}. The peak at m/z 147 and 148 are due to the fragments of the cinnamoyl moiety of **H-3b**. The fragmentation is given in **scheme: 3.5**



Scheme: 3.5

The base peak at m/z = 44 is due to the fragment of the acetyl moiety of the compound **H-3a**. See **scheme 3.4**.

Thus PMR signals at the aromatic and the acetyl methyl region, and other ^{1}Hnmr , ^{13}Cnmr spectral data allowed the complete structures of the compounds in **H-3** to be assigned as a mixture of 22 - Hydroxytaraxeryl acetate (22 - Hydroxytaraxer-14-en-3β-ol- acetate), **H-3a**, and 22 - Hydroxytaraxeryl - c*is* - p - coumarate (22 - Hydroxytaraxer - 14- en- 3β- ol-c*is*-p-coumarate), **H-3b**.

ISOLATION AND CHARACTERIZATION OF A NEW TARAXERYL ESTER WITH AN ADDITIONAL – OH FUNCTIONALITY

Jose E.A " Phytochemical evaluation of some plants used in folk medicine" Thesis. Department of Chemistry , University of Calicut, 2002

CHAPTER – IV

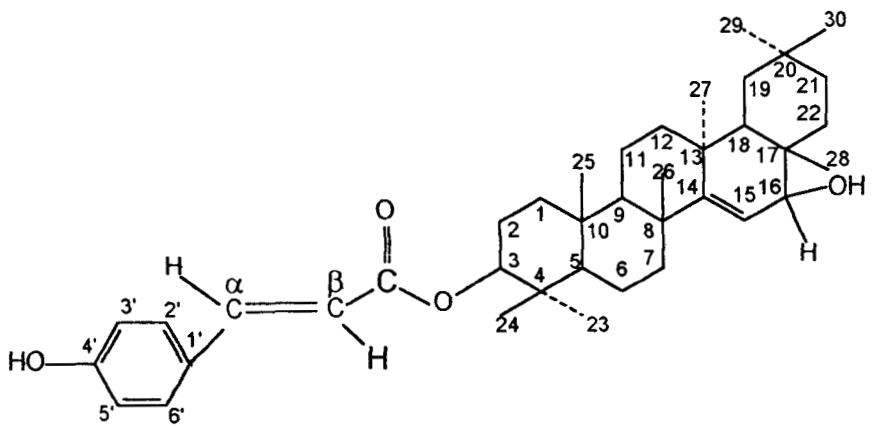
ISOLATION AND CHARACTERIZATION OF A NEW TARAXERYL ESTER WITH AN ADDITIONAL - OH FUNCTIONALITY

Taraxeryl acetate and coumarate with an additional -OH functionality at Carbon-22 has been obtained as the second and third fraction from the column chromatographic separation of the extract of leaves of *Hibiscus schizopetalus*. On further elution of the column with more polar solvent gradient gave two compounds as fraction four **H-4** and five **H-5**. In this chapter characterization and structural elucidation of the fifth fraction **H-5** is discussed. This change in the elution order is brought because this compound **H-5** which is obtained as the fifth fraction also is an ester of taraxerol with an additional hydroxyl functional group. This also is a new compound which has not been reported earlier.

The compound **H-5** was obtained on elution of the column with petroleum ether and ethyl acetate combination in the ratio 3:1.

The solvent was evaporated off to get a slightly greenish coloured substance. This was further purified by preparative TLC and then recrystallized from acetone gave crystals with greenish tinch. It melted at 260-262^{0}C. The compound was then subjected to colour reactions with Liebermann-Burchard, Vanillin -Sulphuric acid, Anisaldehyde-sulphuric acid reagents etc and answered positive. Hence it can be assumed that it is a triterpenoid. It decolourised alkaline potassium permanganate indicating unsaturation in the molecule. It also gave a bluish green colouration with neutral ferric chloride solution. Hence the presence of phenolic-OH group is identified. It was subjected to TLC analysis using different solvent systems and obtained only single spot proving the homogeneity of the compound. Moreover the melting point also was almost sharp.

Based on IR, ^{1}Hnmr, ^{13}Cnmr and mass spectral data investigations and also on the colour reactions with various spray agents on TLC, The compound **H-5** was identified as 16-Hydroxytaraxer-14-en-3β-ol-*trans*-p-coumarate (16-Hydroxytaraxeryl- *trans*-p-coumarate)



H-5

The IR spectra of the compound showed the presence of an ester Carbonyl at 1705 cm^{-1} indicating that it is an α,β-unsaturated ester. It also gives alcoholic O-H stretch, a broad peak between 3600-3200 cm^{-1} region. Absorptions are present both at aromatic and aliphatic C -H stretch 3194cm^{-1} & 3051 cm^{-1} and 2934cm^{-1} & 2858 cm^{-1} respectively. The absorption at 1608.5 cm^{-1} can be assigned to aromatic C=C stretching vibration. It also gives medium absorption at 1514 cm^{-1}, 1458 cm^{-1}, 1379 cm^{-1} etc. indicating the presence of aliphatic C = C stretching and C-H bending. The absorption band at 1267 cm^{-1} can be assigned to C-O-C stretching vibration. The fingerprint region gives characteristic absorption bands.

The FAB Mass spectrum of the compound did not give the molecular ion. The ^{13}C-NMR/DEPT spectra shows 39 distinct Carbon peaks showing that the compound contains 39 Carbon atoms. But from the FABMS no molecular ion matching to the molecular weight of the compound is seemed to be recorded. The mass spectrum shows the mass region recorded only up to 550.

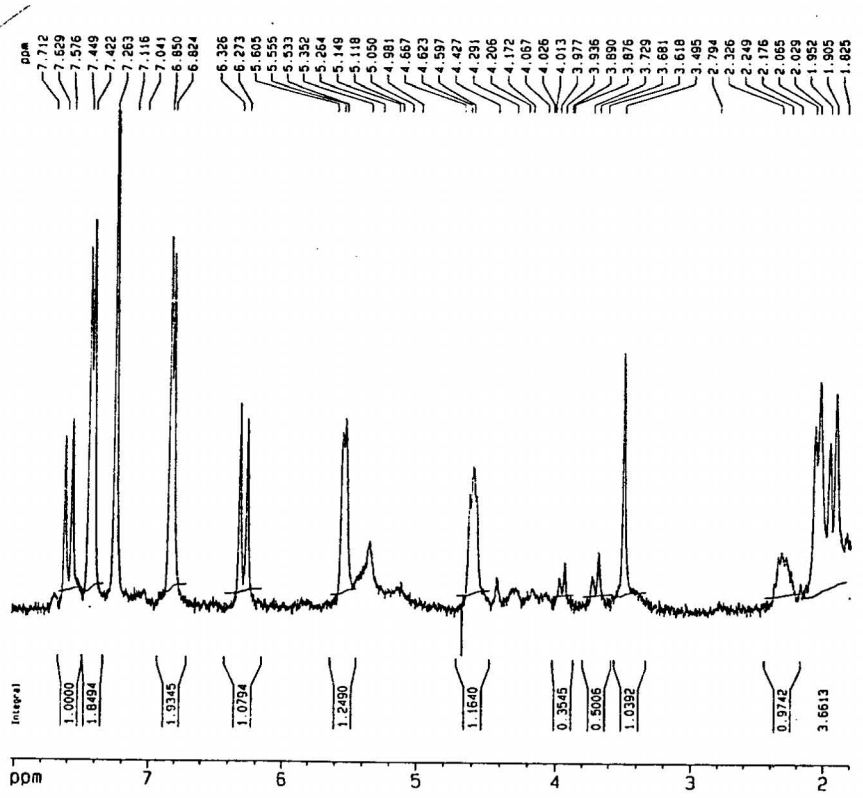
The ^{1}H- and ^{13}C- NMR spectra exhibited a pentacyclic triterpenoid pattern. In the ^{1}H-NMR spectrum eight peaks were obtained between δ 0.826 to δ1.105 as singlets. These are assigned to the eight tertiary methyl groups. The ^{13}C spectra and DEPT edited spectra confirms this

observation. The DEPT-90^0 spectrum contains twelve positive signals which forms the signals for ten CH (methine) groups and other peaks can be taken as artifacts. In DEPT-135^0 spectrum a total of nine negative peaks are detected indicating nine CH_2 groups. Also, DEPT-135^0 spectrum contains 20 positive peaks which forms the signals for the CH_3 and CH groups. In both DEPT -90^0 and -135^0 spectra some artifact signals are also present (eg:- 32.8 ppm, 22.7 ppm, 29.9 ppm, 131 ppm)

In the PMR spectra the tertiary methyl groups appeared as singlets at δ0.826, δ0.914, δ0.981, which accounts for three and the signals at 0.914 and δ0.956 are due to 4 methyl groups which are attributed to the gem dimethyl group at C-4 and C-20. Another singlet at δ1.105 which also integrated for three protons constitute the eighth methyl group. The presence of -C = C**H**- C**H**OH is indicated by the peaks at δ5.544 (1H, d, J=6.6Hz) and δ4.026 (1H, d, J=4Hz). Both the signals appeared as doublets. The ^{1}H signals at δ5.544 (1H, d,J=6.6Hz)and δ4.026 (1H, d, J=4Hz) were assigned for olefinic H at C-15 and H at C-16 respectively as found in many pentacyclic tripterpenes[145]. In the ^{13}C-NMR spectra the signal at 158 ppm can be attributed to C-14 carbon. The presence of the-OH group can be understood by the presence of the broad IR absorption centred at 3356cm^{-1}. The ^{1}Hnmr and ^{13}Cnmr spectra also confirms this observation. The -OH group is placed at the C-16 and the -O**H** proton signal observed as a singlet at δ3.495. The position of the -OH group is

given at C-16 based on the splitting pattern of ^{1}H signal of the hydrogen attached toC-15 and C-16 carbons. The PMR signal at δ5.544 for the hydrogen on C-15 was appeared as a doublet with a coupling constant J=6.6 Hz showing that this interacts with C-16 hydrogen. Also, the PMR signal at δ4.026 for the hydrogen on C-16 was obtained as a doublet with a coupling constant J = 4 Hz showing that it interacts with the C-15 hydrogen. The appearance of ^{1}H signals for Hydrogen atoms at C-15 and C-16 carbons as doublets could be possible only if the OH group is placed at C-16[156,157]. The ^{13}C signals at 117 ppm and 69.7 ppm are attributed to C-15 and C-16 carbons of taraxeryl skeleton[138]. For C-3 which also is attached with an oxygen functionality is assigned the ^{13}C signal 81 ppm. The proton attached to this is identified as having a δvalue of 4.597 ppm which is obtained as a multiplet. The miltiplet formation can be justified because it interacts with the hydrogen on neighbouring carbon atoms.

Inspection of the aromatic and olefininc region of the ^{1}Hnmr and ^{13}Cnmr data of the compound (see **table 4.1**) suggested that it contains a para-disubstituted benzene ring and a *trans*-disubstituted olefinic group. The ^{1}H-NMR signal at δ7.436 (2H, d, J = 8.1 Hz) and δ6.837 (2H, d,J=8.1 Hz) both integrated for two protons each with a coupling constant J = 8.1 Hz could be ascribed to 2', 6' and 3', 5' protons of the phenyl ring

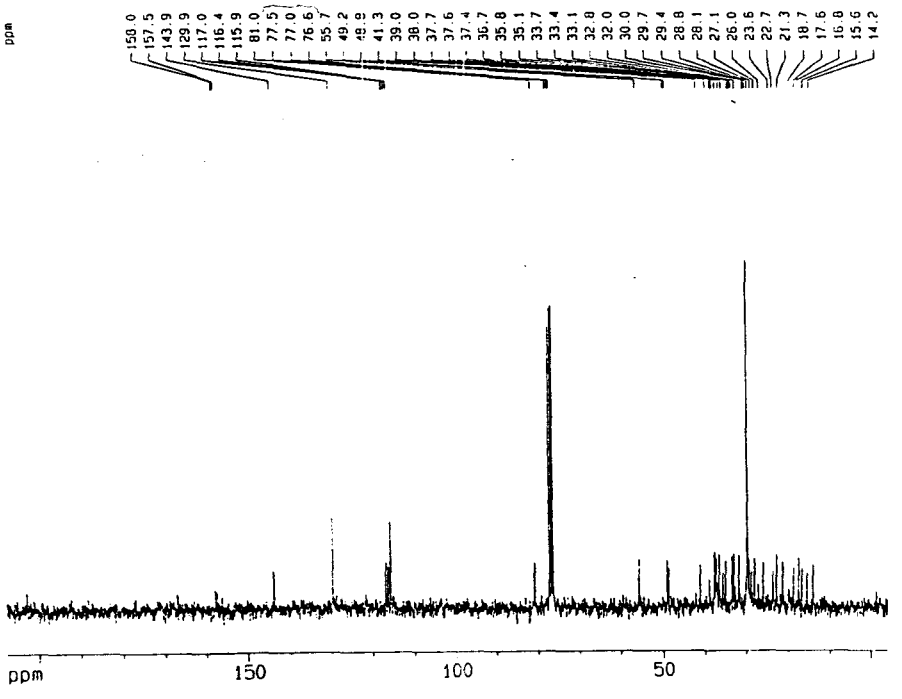


^{1}H-NMR spectrum of H-5 (1.8 - 8 ppm)

respectively. In ^{13}C-NMR spectra the signals at 129.9 ppm and 115.9 ppm are assigned to the carbon atoms at 2',6' and 3',5' respectively of the phenyl ring. The two signal in the ^{1}H-NMR at δ7.602 (1H, d, J=15.9 Hz) and δ6.3 (1H, d, J = 15.9 Hz) are due to the two α and β-olefinic hydrogen. From the value of coupling constants it can be assumed that it is *trans* substituted. The ^{13}C peaks for the carbon at this positions are assigned the value 143.9 ppm and 116.4 ppm respectively. The phenolic

OH proton signal in the PMR is at δ5.264 and the phenolic OH carrying carbon is identified as the signal at 157.5 ppm in the ^{13}C-NMR spectrum. These observations are in agreement with the confirmation that there is a p-substituted *trans* coumaroyl ester moiety present in the compound. Also all ^{1}H & ^{13}C-NMR values are in accordance with the reported taraxeryl - *trans* - p - coumarate and other pentacyclic triterpenes[138,152,158].

The only disagreeing factor is the absence of ^{13}C peak for -CO_2-of the coumaroyl moiety. If it would appear, its δc should have been at 167-170 ppm. This absence may be due to the low intensity of the signal and insufficient amount of the sample.



^{13}C-NMR spectrum of H-5

The ^{1}H- and ^{13}C -NMR assignments for the compound **H-5** are given in **Table 4.1**.

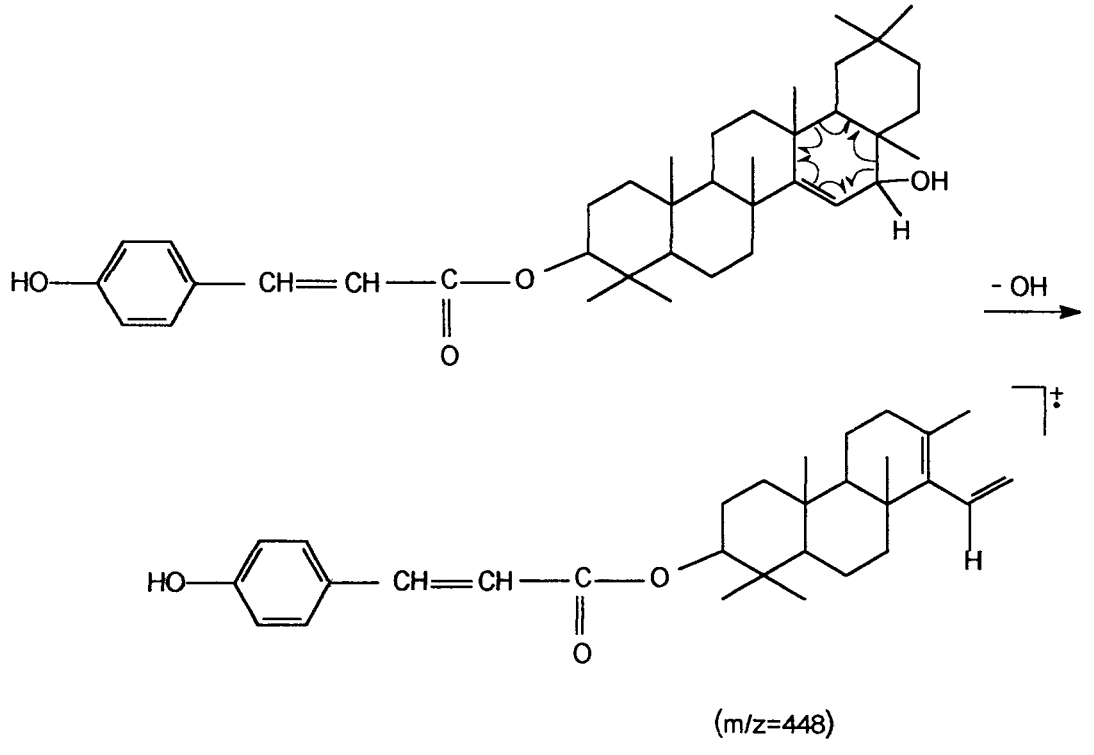
Table - 4.1

^{13}C-and ^{1}H-NMR assignments (recorded in $CDCl_3$) for compound H-5

Carbon[1]	DEPT	δc	δH	Carbon[1]	DEPT	δc	δH
1.	CH_2	37.4	1.258	21.	CH_2	33.1	1.369
2.	CH_2	23.6	1.584	22.	CH_2	35.1	1.48
3.	CH	81	4.597(m)	23.	CH_3	28.1	0.914 (s)
4.	C	38	---	24.	CH_3	16.8	0.956 (s)
5.	CH	55.7	1.258	25.	CH_3	15.6	.981 (s)
6.	CH_2	18.7	1.369	26.	CH_3	26	1.105 (s)
7.	CH_2	41.3	1.48	27.	CH_3	21.3	0.914 (s)
8.	C	39	---	28.	CH_3	29.7	0.826 (s)
9.	CH	49.2	1.328	29.	CH_3	33.4	0.956 (s)
10.	C	37.14	---	30.	CH_3	30	0.914 (s)
11.	CH_2	17.6	1.48	1'	C	128.9	---
12.	CH_2	33.7	1.369	2',6'	CH	129.9	7.436 (d)
13.	C	37.59	---	3',5'	CH	115.9	6.837 (d)
14.	C	158	---	4'	C	157.5	----
15.	CH	117	5.544(d)	α	CH	143.9	7.602 (d)
16.	CH	69.7	4.026(d)	β	CH	116.4	6.3 (d)
17.	C	35.8	---	Phenolic **OH**	---	---	5.264
18.	CH	48.8	1.328	C=CH- **OH**	---	---	3.495
19.	CH_2	36.7	1.369	- CO_2 -	C	---	---
20.	C	28.8	---				

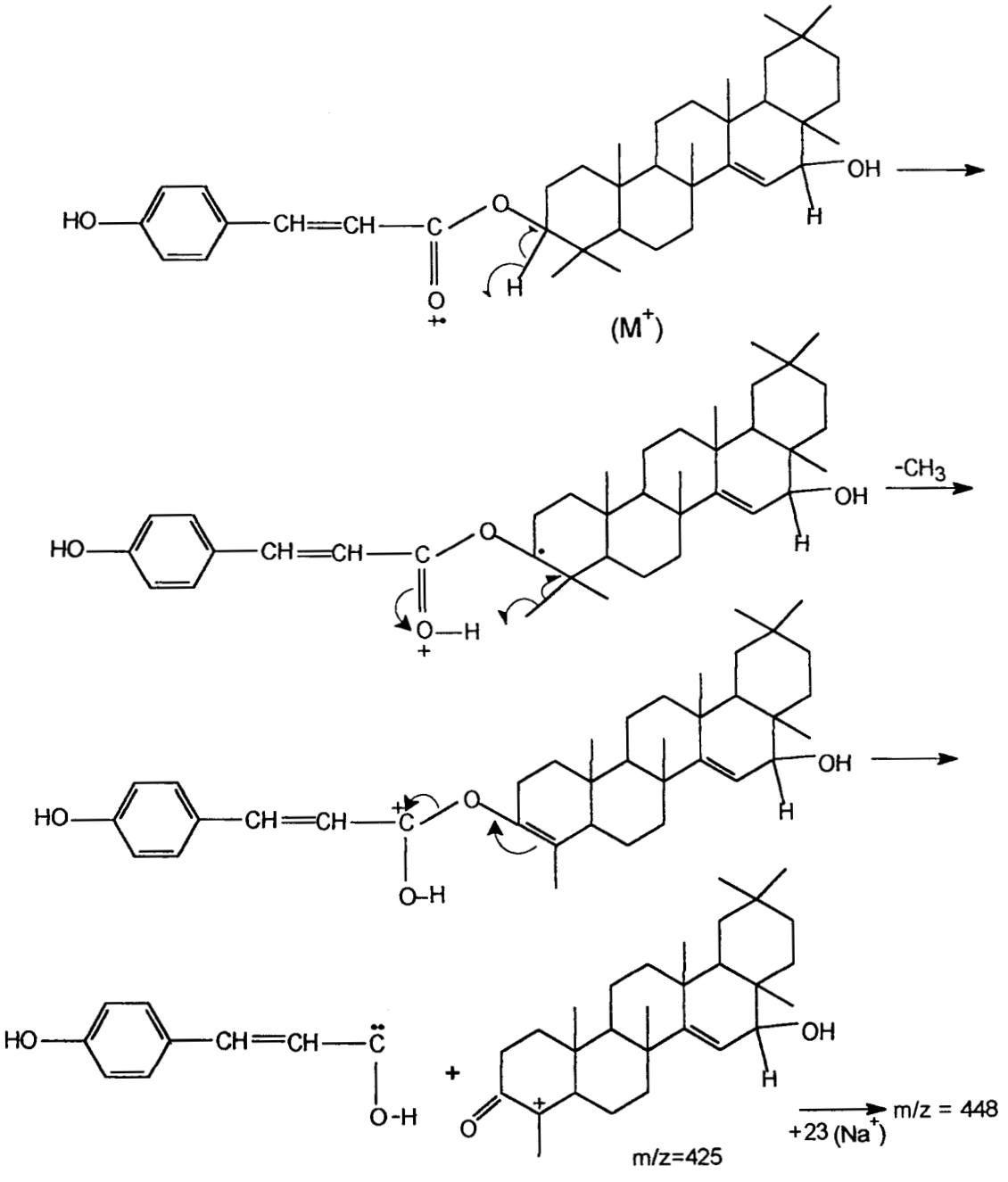
[1]. Numbering as shown in structure for **H-5**

The mass spectrum of the compound did not give the molecular ion peak. The spectrum was recorded only up to a mass range of 550. The absence of molecular ion peak in the mass spectrum may also be explained due to the presence of an -OH group at C-16, adjacent to an olefinic bond. Such a molecule will be highly unstable and would undergo decomposition. From the suggested structure of the compound the molecular mass could be calculated as 588. The peak with highest m/z value in the spectrum is at m/z 448. The formation of this ion can be explained by a retro -Diels - Alder fragmentation path way[149,150,151] characteristic of pentacyclic triterpenoids. The formation can be depicted as given in **Scheme: 4.1**.



Scheme: 4.1

This is very much helpful in establishing the position of the double bond and nature of the lower part of the molecule. Alternatively, the formation of the peak at m/z = 448 can also be explained as given in **Scheme: 4.2.**

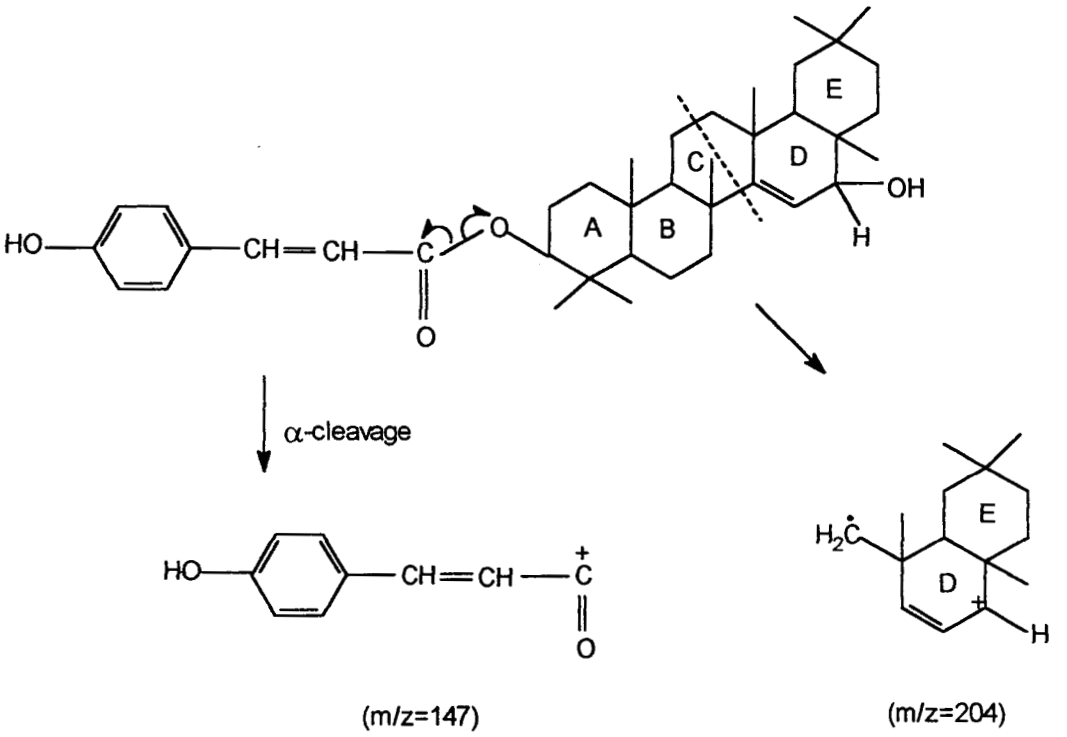


Scheme: 4.2

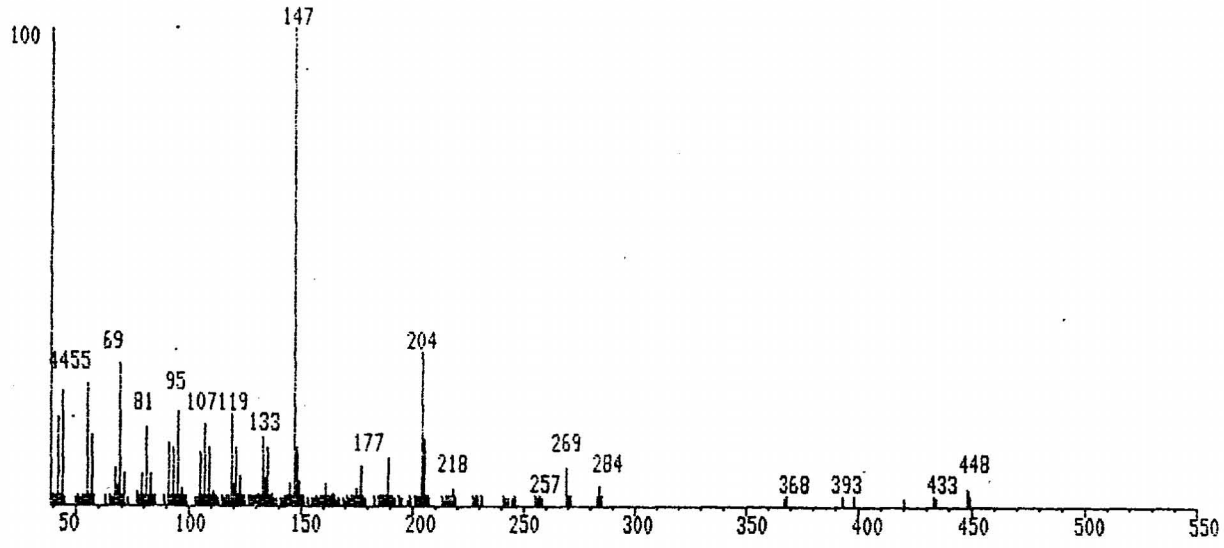
Addition of mass units of 23,39 etc are commonly found on FAB mass spectrum by addition of alkali metal ion from the matrix.

The other ion at m/z 433 (448-15) can be described by the consecutive loss of methyl group from the ion at m/z 448.

The other peaks at m/z 204 and 147 are also in accordance with the proposed structure of compound **H-5**. The base peak at m/z 147 is due to the fragment of the para hydroxy cinnamoyl moiety. The fragmentation[147,149] is given in **Scheme: 4.3**.



Scheme:4.3



FAB Mass spectrum of H-5

Consideration of ^{1}Hnmr and ^{13}Cnmr data as well as IR Mass spectral data allowed the complete structure of the compound to be assigned as 16-Hydroxytaraxer - 14 - en - 3β - ol - *trans* - p - coumarate (16- Hydroxytaraxeryl-*trans*-p-coumarate) with the molecular formula $C_{39}H_{56}O_4$.

ISOLATION AND CHARACTERIZATION OF A NEW STEROIDAL TRITERPENOID FROM HIBISCUS SCHIZOPETALUS

Jose E.A " Phytochemical evaluation of some plants used in folk medicine" Thesis. Department of Chemistry , University of Calicut, 2002

CHAPTER V

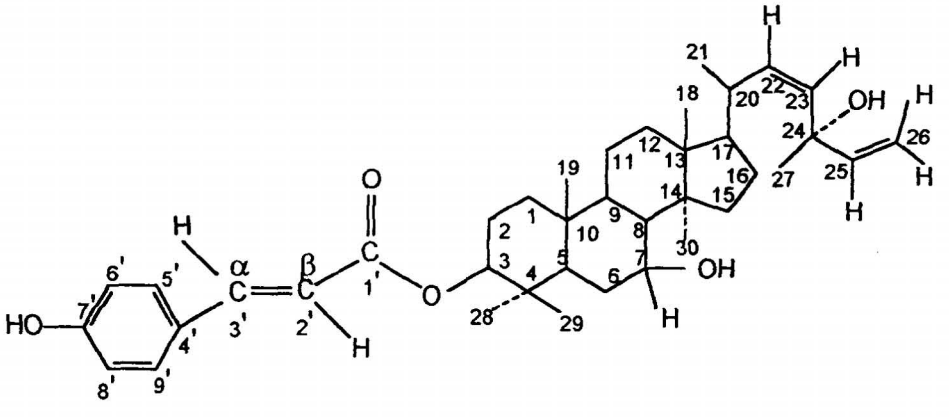
ISOLATION AND CHARACTERIZATION OF A NEW STEROIDAL TRITERPENOID *FROM HIBISCUS SCHIZOPETALUS*

Lanosteroids are steroidal compounds of plant origin. Compounds with Lanostane skeletal structure such as lanosterol and highly oxygenated lanostanes are bestowed with a variety of useful biological activities. Large volume of works have been done on the immuno stimulant, anti-allergic, anti-cancer, anti-hypertensive and anti-inflammatory activities of lanostane derivatives[160,161].

The column on elution with petroleum ether-ethyl acetate (4:1) gave compound **H-4** as the fourth fraction. The solvent on evaporation gave a pink coloured amorphous substance. It was further purified by preparative TLC and recrystallized from benzene-chloroform mixture (1:2). The pink crystals thus obtained melted at 193-195^{0}C. On thin layer chromatography it gave only a single spot indicating that it is pure. The

compound gave positive colour reactions of steroid type compounds. It gave blue green spot spraying with Liebermann-Burchard reagent. With Vanillin-Sulphuric acid reagent the compound gave a blue colour. The examination with Anisaldehyde-Sulphuric acid reagent the compound gave blue-violet colour, and pink colour with 20% Sulphuric acid. It decolourised Baeyer's reagent indicating unsaturation in the molecule.

Based on the detailed investigation of available spectral data, such as IR, ^{1}H- and ^{13}C-NMR and FABMS, the structure for the compound **H-4** is proposed as 7,24-Dihydroxylanosta-22,25-dien-3β-ol-*trans*-p-coumarate.

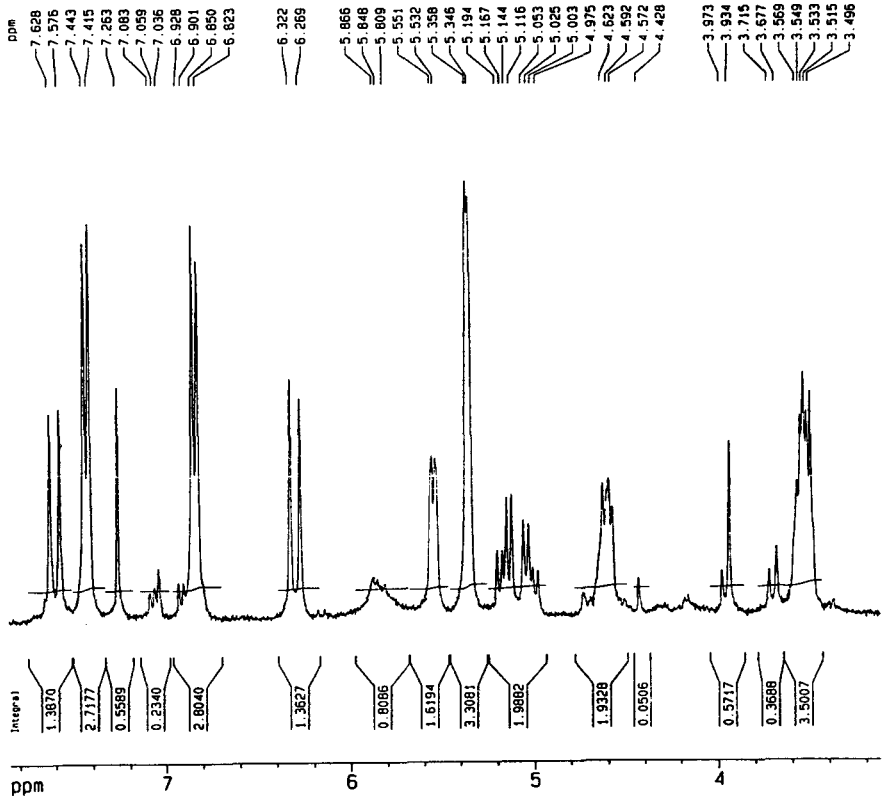


H-4

IR spectroscopy of the compound shows a strong broad absorption between 3600-3270cm^{-1} indicating the presence of hydroxyl groups. It showed characteristic absorption both in the aromatic and aliphatic regions. The presence of a Carbonyl function is indicated by the absorption at 1707cm^{-1}. From the ν value it can be understood that this is due to an α, β-unsaturated ester function. Absorptions are also occurred at aromatic C=C str and C-O str regions (1676 cm^{-1} and 1606 cm^{-1}). Other prominent peaks are at ν 1514cm-1, 1461.9cm^{-1}, 1267cm^{-1}, 1172cm^{-1}, 1107cm^{-1} and 1047.3cm^{-1}, which are characteristic absorption peaks for CH_2 bending, aliphatic C=C stretching. It also showed bands at 2937.4cm^{-1} and 2860.2 cm^{-1} which was due to C-H str of –CH2 (asym & sym). Apart from this, the finger point region carries other characteristic peaks of a tetracyclic triterpenoid type compound.

Examination of the ^{1}H-NMR spectra reveals the presence of p-coumaroyl moiety in the compound[138,158]. The ^{13}C-NMR data also confirms this observation. The compound is a triterpene, and most probably a tetracyclic one, with a Lanostane skeletal structure. Spectral data also indicates the presence of two free hydroxyl groups and two double bonds. Both the two double bonds are in the side chain attached to position 17 of the tetracyclic skeleton. The relevant signals of the p-coumaroyl groups are seen in the ^{1}H- and ^{13}C-NMR spectra as follows.

The ∝-olefinic hydrogen (that is the one away from the Carbonyl group) gives a signal as a doublet at δ 7.60 with a coupling constant J=15.6 Hz and the β-olefinic hydrogen (that is the one next to the Carbonyl group) gives a peak at δ 6.30 as a doublet with a J value of 15.6 Hz. The ∝,β-hydrogen atoms are *trans* to each other as is evident from the coupling constant value. The 6' and 8' hydrogen of the aromatic ring gave the signal at δ 7.43 as doublet with J=8.4 Hz and the 5' and 9' hydrogen gave the signal at δ6.92 as doublet with a coupling constant value J=8.4 Hz. The para hydroxy proton (7') is assigned a value at δ 5.809, a broad peak.



^{1}H-NMR spectrum of H-4 (3.2 - 7.8 ppm)

The corresponding signals in the ^{13}C-NMR spectrum are seen respectively at 144.1 ppm (the ∝ - olefinic Carbon), 130.00 ppm (5',9' Carbons), 115.9 ppm (6',8'Carbons) and 116.3 ppm (the β-olefinic Carbon). The DEPT – 90^0 spectrum also confirms that each of these Carbon atoms carries a hydrogen atom. The 7'-Carbon (the one carrying the phenolic-OH group) is seen in the ^{13}C-spectra at 157.8 ppm. The signal at 158 ppm can be assigned to carbonyl carbon (1') of the ester moiety. The complete assignment of ^{1}H- and ^{13}C-NMR spectral values are given in **Table 5.1** and the numbering of the atoms are given in the proposed structure **H-4**.

The ^{13}C-NMR gives signals for a total of 37 Carbon atoms. (The ^{13}C-spectrum contains some additional signals which can be treated as artifacts. These signals can be deducted by close scrutiny of the decoupled ^{13}C-spectrum and the DEPT spectra). The p- coumaroyl ester has seven carbons (for 5',9' and 6',8', it gave one signal each) and, hence, the number of Carbon in the triterpenoid moiety is 30. Both the ^{1}H- and ^{13}C-NMR pattern shows that it is a tetracyclic terpene. The ^{13}C and DEPT spectra reveals the presence seven methyl carbons, eight methylene carbons and ten methine carbons, of which two has attached to oxygen functionality and three are Sp^2 hybridised. Six Sp^2 hybridised CH carbons constitute the p-coumaroyl moiety which gave four signals between 115.9

ppm to 130 ppm.Out of the seven methyl groups one is gem dimethyl and three are angular methyl groups. The gem dimethyl groups are at carbon atom, C-4 (C-28 & C-29) and the angular methyl groups are at positions C-10, C-13 and C-14. The shift values are given in **Table 5.1**. The other two methyl groups are located in the side chain at C-17 of the lanosterol skeleton as shown in the proposed structure, i.e. C-21 and C-27.

The proton and carbon nmr spectra also shows the presence of two double bonds in the side chain, one of which is terminal and vinyl as suggested by the following signals in the proton nmr spectra[162,163]. Two IH double doublets are seen at δ5.156 and 5.015 with J values of 8.4 Hz (cis or geminal coupling) and J = 15.3 Hz (*trans* coupling). The other terminal olefinic hydrogen of this functionality is seen at δ4.58 as a double doublet (J = 6,9.3 Hz, cis and geminal coupling). To account for the multiplicity of the vinyl protons, a hydroxyl group should be attached on the carbon (oxygenated quaternary carbon) next to the mono substituted vinyl carbon. Vinyl carbons are numbered C-25 and C-26. The hydroxyl attached carbon has the number C-24. This carbon also carries a methyl group C-27. In addition to the tertiary hydroxyl this compound probably has another hydroxyl group, a secondary one, as suggested by the multiplet at δ 3.549 attributable to a hydrogen attached to a carbon carrying one hydroxyl group. The position of this hydroxyl

group is not clear, but tentatively it can be placed at position 7 on biosynthetic considerations. In the ^{13}Cnmr spectrum, the vinyl carbons are seen at 138.4 ppm (mono substituted carbon) and 117 ppm (terminal carbon). The other two side chain olefinic carbons are seen at 121.9 ppm and 129.4 ppm. The corresponding proton signals for these two olefinic protons are at δ 5.41 and 5.352, each as doublet with J values of 5.7 Hz and 3.6 Hz respectively due to vicinal and allylic coupling. There is no apparent coupling between the two olefinic hydrogen due to the very small difference in chemical shifts. The DEPT spectra also are in conformity with these observations. However, the structure suggested is tentative. To have a conclusive evidence a correlation spectral studies are required. Therefore, the sample has been sent for HMBC and COSY spectra and the results are awaited. Due to lack of time, with the available evidence the tetracyclic triterpenoid structure with a p-coumaroyl functionality has been suggested and the discussion presented.

The ^{1}H- and ^{13}Cnmr assignments[164] for the compound **H-5** are given in **table 5.1.**

Table - 5.1

^{13}C- and ^{1}H-NMR assignments[1] (recorded in $CDCl_3$) for compound H-4

Carbon[2]	DEPT	δc	δH	Carbon[2]	DEPT	δc	δH
1.	CH_2	35.1	1.258(m), 1.445	21.	CH_3	19.43	1.032
2.	CH_2	33.12	1.215(m) 1.284	22.	CH	121.9	5.41(d)
3.	CH	81	3.948(m)	23.	CH	129.4	5.352(d)
4.	C	37.7	---	24.	C	76*	---
5.	CH	50.2	1.531	25.	CH	138.4	5.156(dd)
6.	CH_2	37.4	1.901(m), 1.954	26.	CH_2	117	5.015(dd) 4.58(dd)
7.	CH	72	3.549(m),	27.	CH_3	28.1	1.105
8.	CH	36.2	1.369	28.	CH_3	18.7	1.031
9.	CH	56	1.215	29.	CH_3	21.3	0.985
10.	C	38	---	30.	CH_3	17.6	0.931
11.	CH_2	26	1.566, 1.626	1'	C	158	---
12.	CH_2	31.91	1.147, 1.654	2'(β)	CH	116.3	6.3(d)
13.	C	42.3	---	3'(α)	CH	144.1	7.6(d)
14.	C	37.3	---	4'	C	127.3	----
15.	CH_2	31.7	1.180, 1.901	5',9'	CH	130	6.92(d)
16.	CH_2	28.3	1.160, 1.829	6',8'	CH	115.9	7.43(d)
17.	CH	55.8	1.481	7'	C	157.8	---
18.	CH_3	19.86	0.914	Phenolic **OH**	---	---	5.809(br)
19.	CH_3	19.06	1.009	Alcoholic **OH**	---	---	4.428
20.	CH	29.2	1.326				

1. Data assigned by comparison with Tanaka et al[164]

2. Numbering as shown in structure H-4

* The signal might have been formed in the region 74-77 ppm which possibly is marked by the $CDCl_3$ peak.

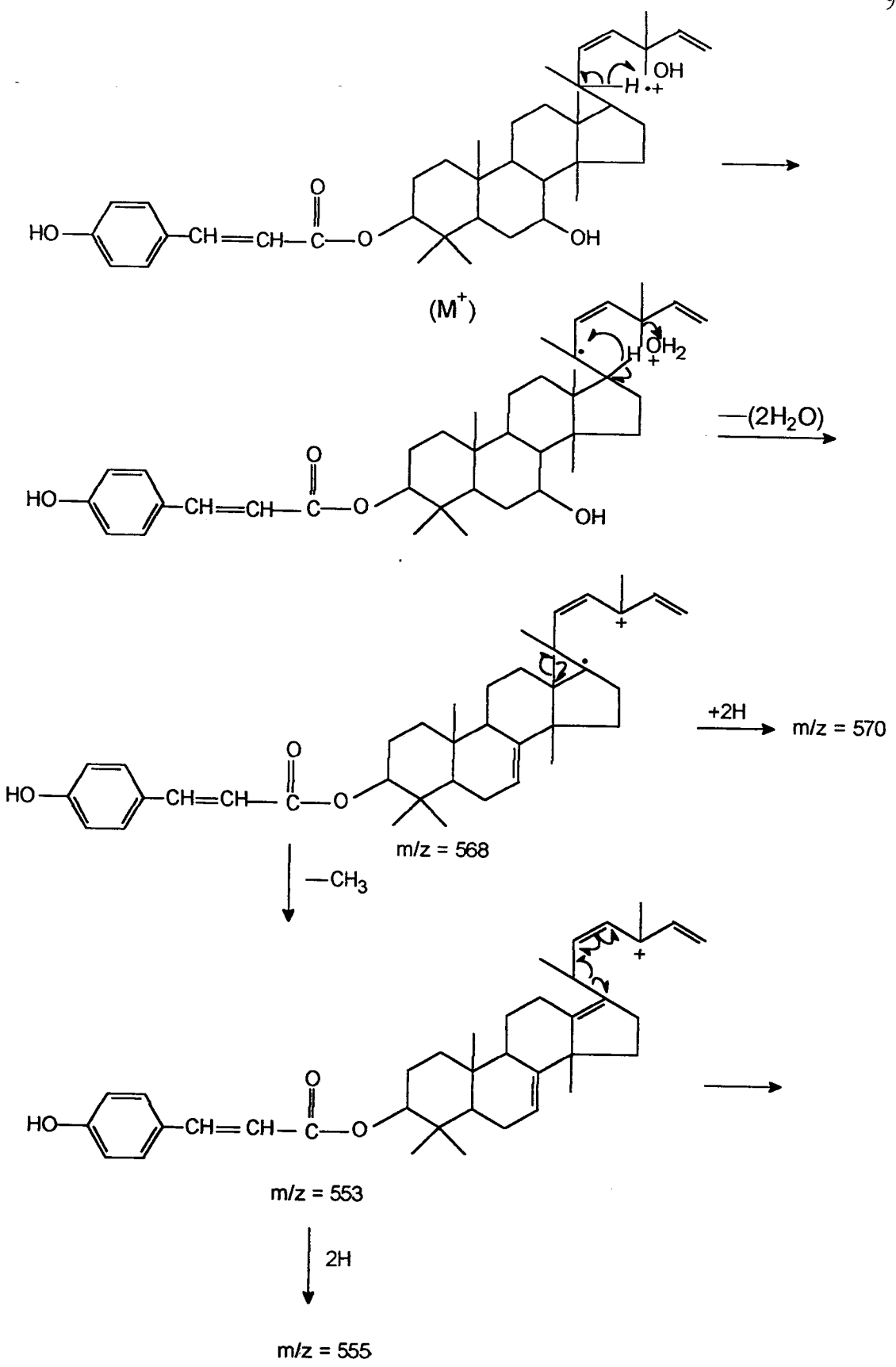
The proposed structured could also be explained on the basis of mass spectral pattern of the compound. The FAB mass spectrum of the

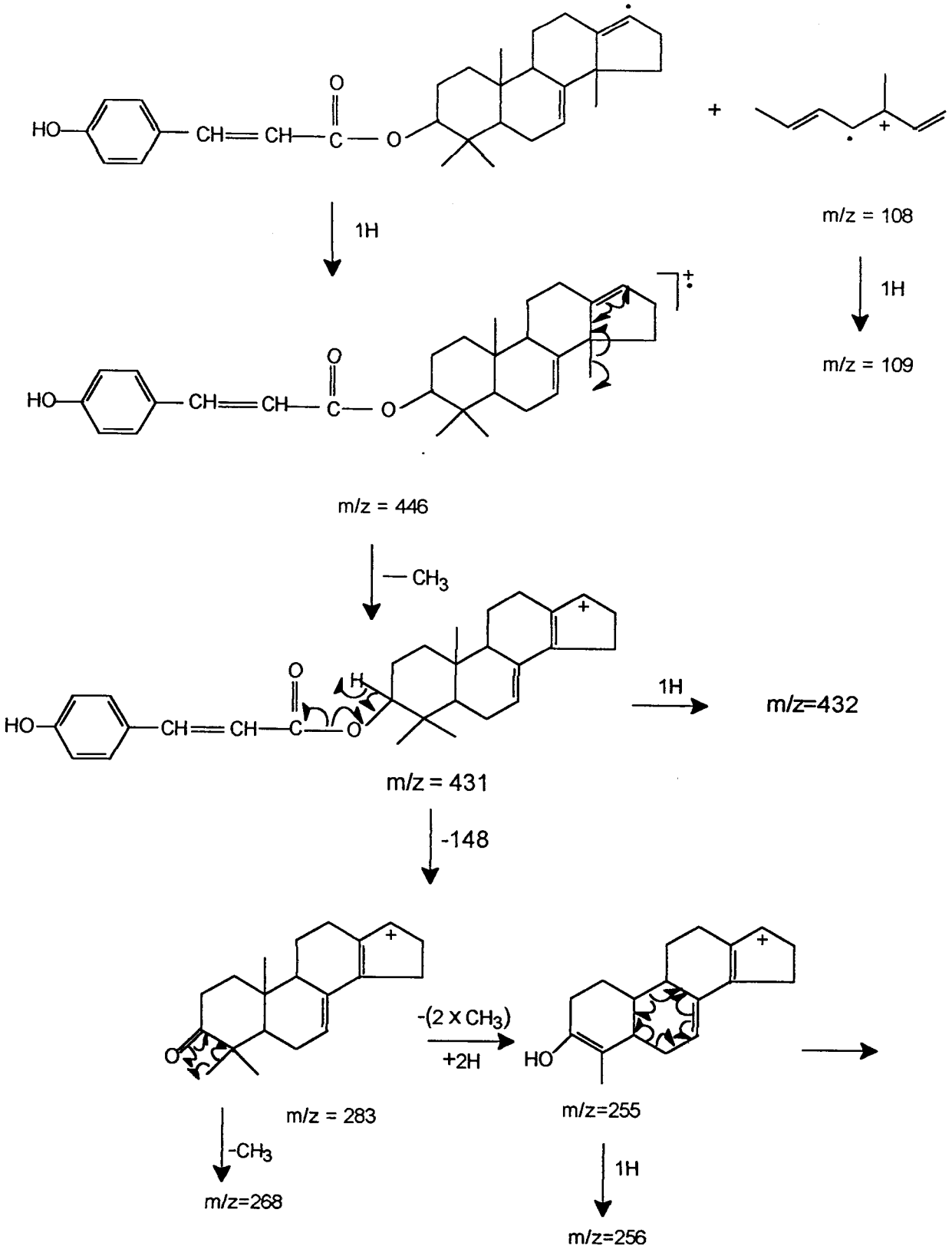
compound did not show molecular ion peak. From the proposed structure of the compound the molecular mass could be calculated as 604. Then the expected molecular ion peak should have been at m/z 604. But the spectrum was recorded only upto a mass range of 600. The fragmentation pattern of steroids are complex involving complicated hydrogen *trans*fer rearrangements. The peak in the mass spectrum can be explained as depicted in the schemes given below.

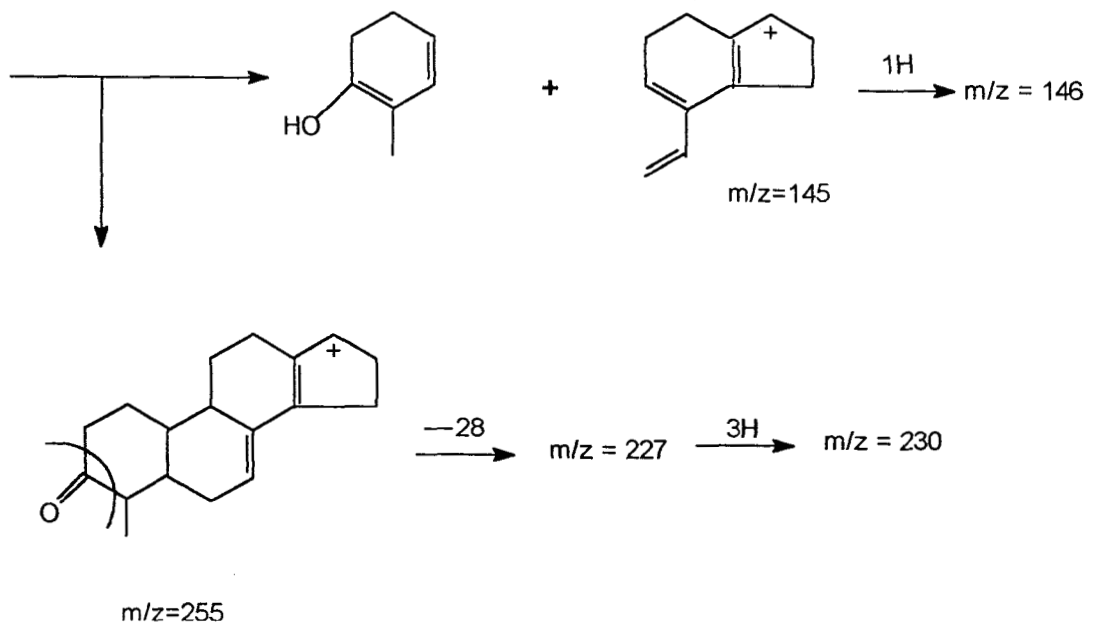
The peak with highest m/z value at 570 might have been formed by the loss of two water molecules. The peak at m/z 555 is due to the loss of one methyl group from the ion m/z 570 (570-15=555), which on side chain fragmentation gave the peak at m/z 446 and 109. The loss of one or more angular methyl groups and side chain fragmentation from the molecule is a common pattern in methylated steroidal structures. The formation of ions at m/z 432, 283, 268, 256,230,146 etc can be explained as the successive loss of methyl, coumaroyl and again methyl groups from the ion at m/z 446. The fragmentation pattern is given in **scheme 5.1**. The formation of peak at m/z 476 can be represented as -

$[M^+\text{-}R(125)] \longrightarrow$ m/z 479 $\xrightarrow{-3H}$ m/z 476, where

R = side chain

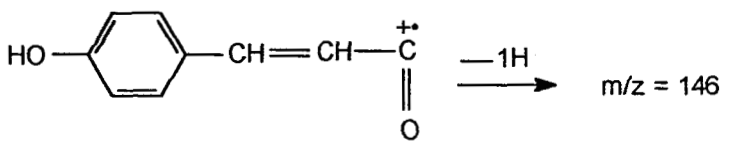

HO
CH=CH
C
O
OH
(M^+)
OH_2
$-(2H_2O)$
+2H
m/z = 570
m/z = 568
$-CH_3$
m/z = 553
2H
m/z = 555


HO
CH
CH
C
O
O
+
m/z = 108
1H
1H
m/z = 109
m/z = 446
— CH3
1H
m/z=432
m/z = 431
-148
-(2 x CH3)
+2H
HO
m/z = 283
m/z=255
-CH3
1H
m/z=268
m/z=256



Scheme:5.1

The base peak at 146 can also be obtained from coumaroyl moiety.



Thus, all the peaks obtained in the FAB mass spectrum are quite consistent with the proposed formula for compound **H-4**.

Consideration of IR, ^{1}H-& ^{13}C-NMR and mass spectral data, the compound **H-4** has been characterized as 7,24-Dihydroxylanosta-22,25-dien -3β-ol-*trans*-p-coumarate with the molecular formula $C_{39}H_{56}O_5$. However, for an unambiguous characterization of structure HMBC and COSY correlation spectral studies are required.

EXPERIMENTAL
- Phytochemical Examination of Hibiscus schizopetals

Jose E.A " Phytochemical evaluation of some plants used in folk medicine" Thesis. Department of Chemistry , University of Calicut, 2002

CHAPTER – VI

EXPERIMENTAL

- Phytochemical Examination of *Hibiscus schizopetals*

Hibiscus schizopetalus of Malvaceae family is one of the least phytochemically examined species of genus, Hibiscus. The Secondary metabolites obtained from the leaves of the plant were characterized by chromatographic as well as spectroscopic methods. The characterization and identification of the products **H-1, H-2, H-3, H-4** and **H-5** obtained from *H.Shizpetalus* supports the proposal that Taraxeryl and Lanosteryl esters are characteristic of the genus Hibiscus.

VI.1. General Experimental Procedures

Plant Material

The leaves of the plant were collected from Mattom Desam of Aloor village of Thrissur district, Kerala and was authenticated by Dr.A.K.Pradeep of Botany Department, Calicut University, Kerala.

A voucher specimen (No: 70560) has been deposited in the Herbarium [CALI] of Botany Department, University of Calicut.

Melting Point determination

All the melting points of Crystalline isolates were determined using Toshniwal Capillary melting point apparatus and are uncorrected.

Infra - red absorption spectroscopy (IR)

The IR spectra of the isolates were recorded using a SHIMADZU FTIR - 8101-A spectrometer and the spectra were determined in KBr Pellets.

Nuclear Magnetic Resonance Spectroscopy (NMR)

The ^{1}H-and ^{13}C-NMR spectra of the isolates were recorded on a VARIAN UNITY PLUS-300 spectrometer using $CDCl_3$ as solvent with tetra methyl silane (TMS) as internal standard. The chemical shifts are recorded in ppm (δ).

Fast Atom Bombardment Mass Spectrometry (FABMS)

The FAB mass spectra were recorded using a JOEL SX-102 (FAB) mass spectrometer.

Column Chromatography (CC)

Column chromatographic separation of the crude and semi purified extracts were carried out using silica gel (Acme, 100 - 200 mesh). The

columns were prepared as a slurry with suitable solvents and eluted with selected chromatographic solvents.

Thin Layer Chromatographic Analysis (TLC)

Thin layer chromatographic plates were prepared using TLC grade silica gel - G (Acme). For preparative TLC, chromatographic plates were prepared using Stahl apparatus. All solvents used in the course of the work were purified by standard procedures described by 'Vogel'[23]

VI.2. Reagents

Liebermann - Burchard reagent (LB reagent)

Acetic anhydride (5ml) was added carefully to concentrate sulphuric acid (5ml) and this mixture was added to absolute ethanol (50ml), while cooling in ice.

The sprayed plate is heated to 110^0c until maximal visualisation of the spots.

Vanillin - Sulphuric acid reagent (VS reagent)

The reagent was prepared by dissolving Vanillin 1gm in ethanol (100ml) and sulphuric acid (5ml) in ethanol (100ml) separately.

The chromatogram (TLC) was sprayed first with 5% sulphuric acid, followed immediately by 1% ethanolic Vanillin. The sprayed plate is then heated at 110^0C for 5 - 10 minutes until maximal visualisation of the spot.

Anisaldehyde - Sulphuric acid reagent (A S reagent)

Anisaldehyde (0.5ml) was mixed with glacial acetic acid (10ml) and diluted with methanol (85ml) and concentrated sulphuric acid (5ml) was added to it and mixed.

The TLC place was sprayed with about 10ml, heated at 100^0C for 5 - 10 minutes until maximal visualisation of the spots were obtained.

20% Aqueous Sulhuric acid (20% H_2SO_4)

20% aqueous Sulphuric acid is prepared. The sprayed plate is heated to 110^0c until spots are visualised.

Baeyer's Reagent

Very dilute alkaline potassium permanganate solution is known as Baeyer's reagent.

The plate is sprayed with the reagent. The unsaturation is indicated by the discharge of pink colour of the Baeyer's reagent.

VI.3. Extration, fractionation and isolation of Compounds from the leaves of *H. Schizopetalus.*

The leaves of *H. Schizopetalus* was collected and dried in shade. About 2 kg of the dry powdered material was extracted repeatedly using a soxhlet extractor with petroleum ether ($60-80^0$c) for 36 hrs. The combined extract was then concentrated under reduced pressure to about

500 ml using a rotary vacuum flash evaporator. This extract was then adsorbed on Silica gel (100 - 200 mesh) and packed above a column of Silica gel (750 gm) in a glass column (3cm X 100 cm). The column was then eluted with petroleum ether, petroleum ether - ethyl acetate mixture of varying compositions (gradient) and then with ethyl acetate alone. 15ml aliquots were collected in test tubes and the contents of each tube was compared by thin layer chromatography. The fractions which gave identical spots were pooled. These fractions were grouped according to their homogeneity judged from the TLC analysis. The petroleum ether - ethyl acetate mixture fractions were combined and subjected further to column chromatography on silica gel (100-200 mesh) and eluted with solvents of increasing polarity viz., petroleum ether, different combinations of petroleum ether - ethyl acetate and ethyl acetate in that order. Several 10ml fractions were collected and each fraction monitored by TLC. Identical portions were pooled together and the solvent evaporated under reduced pressure. Different compounds isolated from the petroleum ether extract of *H. Schizopetalus* were designated as **H-1, H-2, H-3, H-4** and **H-5**. The compound **H-1**, was recrystallized from petroleum ether to yield a colour less substance (90mg) which melted at 41-43^{0}C. The compound **H-2** was recrystallized from benzene - ethyl acetate (3:2) as white powder (100mg) which melted at 250 -252^{0}C. The

compound **H-3** obtained as the third fraction was recrystallized from benzene - ethyl acetate (1:1) to yield a white powder (200mg) which melted at 245 - 247^{0}C. The compound **H-4** was recrystallized from benzene - chloroform mixture (1:2) to yield pink crystals (80mg) which melted at 193-195^{0}C. The compound **H-5** was recrystallized from acetone to yield a slightly greenish coloured crystals (75mg) which melted at 260 - 262^{0}c.

VI.4. TLC Analysis of petroleum ether extract

On a coated TLC plate the solution of the extract in petroleum ether (5μl) was applied as a thin spot. It was then placed in the developing chamber containing petroleum ether - ethyl acetate in the ratio 4:1. When the solvent front reached 1cm below the upper edge of the plate, it was withdrawn and the solvent was allowed to evaporate. Liebermann - Burchard reagent, Vanillin - sulphuric acid reagent, Anisaldehyde - sulphuric acid reagent, 20% sulphuric acid reagent and Baeyer's reagent were sprayed over separate plates developed as above and the coloured spots formed were noted. A total number of 8 -10 spots were obtained in each case of which 5 were identified. The TLC results of the compounds isolated from *H. schizopetatlus* were given in table 2.2 of **chapter II.**

Further purification of the compounds isolated was achieved by preparative TLC.

Preparative TLC

The purification by preparative TLC was carried out on chromato plates (20 x 20cm) using TLC grade silica gel-G as the adsorbent. The plates were coated with thick layers of silica gel-G (1-1.5 mm) using Stahl's apparatus. The compounds to be purified were dissolved in benzene and applied on the coated plate using a micro pipette. It was developed using petroleum ether-ethyl acetate in the ratio 4:1. At the end of the development, the location of the bands of interest was determined by spraying guide strips with a specific reagent. The substance containing zone was scrapped off, collected and eluted with the solvent benzene. The solvent was evaporated off for recovery of the solute. It was then recrystallized from appropriate solvent.

The study of the secondary metabolites from *H.schizopetalus* is further extended with the methanolic extract and the characterization of the compounds obtained are under investigation.

VI.5. *Cis*-Hentriacont-3-ene (H-1)

Elution of the Column with petroleum ether afforded colourless substance of compound **H-1** as the first fraction. It is recrystallized from

n - hexane to yield colourless waxy substance (90 mg); mp - 41-43^{0}C. It did not answer Liebermann - Burchard, Anisaldehyde - Sulphuric acid, Vanillin - Sulphuric acid reagent colour reactions. It decolourised the pink colour of the Baeyer's reagent indicating unsaturation in the molecule. The spectral details are as follows:-

I.R (KBr). ν_{max} (cm^{-1}) : 2920, 2850 (C-H str in -CH_2 & CH_3 (asym & sym), 1629.7 (C = C str), 1379 & 1463.9 (C - H bend).

^{1}H-NMR ($CDCl_3$) (δ) : 0.858-0.900 (-CH_3 groups), 1.256,1.602-1.681 (CH_2 groups),2.009 (CH_2 groups next to the double bond), 5.12 (2H,t,J=7.2 Hz, olefinic protons).

^{13}C-NMR ($CDCl_3$) (δ) : 13.3-40.2 (CH_3 and CH_2 carbons), 123.5 (olefinic carbons).

MS (FAB) m/z : M^+(434), 406, 391, 377, 363, 349, 335

From the above chromatographic and spectroscopic data, the compound **H-1** is identified as *cis*-Hentriacont-3-ene.

VI.6. 22-hydroxy taraxeryl acetate (H-2)

Elution of the Column with petroleum ether and Ethyl acetate combination in the ratio (9:1) yielded compound **H-2** as second fraction. It is further purified by preparative TLC. It is recrystallized from benzene-ethyl acetate mixture (3:2) as white powder (100 mg); m.p- 250 - 252^{0}C.

The compound gave positive colour reactions with LB reagent (pink), VS reagent (violet), AS reagent (blue -violet) and 20% H_2SO_4 (pink).A solution of compound **H-2** gave green coularation with LB reagent (triterpenoid compound). It decolourised the pink colour of Baeyer's reagent indicating unsaturation in the molecule. The spectral data are given as follows:-

IR (KBr) ν_{max} (cm^{-1}) : 3907-3265 (O-Hstr); 2922, 2852.5 (C-H str; asym & sym), 1724.2 (ester carbonyl str), 1649 (-C =C str), 1377.1, 1467.7 (C-H bend), 1028, 1251 (C-O-C str).

^{1}HNMR ($CDCl_3$) (δ) : 0.905 (H_3-23 & 30,S), 0.951 (H_3-24 & 29,S), 0.876 (H_3-25,S), 1.090 (H_3 - 26,S), 0.858 (H_3-27,S) 0.819 (H_3-28,S), 1.254 -1.677 (all - CH_2's & CH's, m), 2.043 (-O-CO-**CH_3**, S), 3.641 (H-22,t, J=6.6Hz), 4.459 (H-3, dd, J = 6.6, 9.6 Hz), 5.122 (O-H,br), 5.532 (H-15, dd, J = 3, 8.1Hz).

^{13}CNMR ($CDCl_3$)(δ) : 81.1(C-3), 158 (C-14), 117(C-15), 81.1 (C-22), 170.9 (-CO_2-), 15.5-55.7 (**C**H,**C**H_2 & **C**H_3 carbons).

MS (FAB) (m/z) : 466 (M^+-H_2O), 451, 407,391,344,343,203.

The compound **H-2** thus identified as 22- hydroxy taraxeryl acetate.

VI.7. 22-hydroxy taraxeryl acetate (H-3a) and 22-hydroxy taraxeryl-*cis*-p-coumarate (H-3b)

The Compound **H-3** was obtained on elution with petroleum ether and ethyl acetate combination in the ratio 85:15 as the third fraction. It is recrystallized from benzene-ethyl acetate mixture (1:1) as white powder (200mg); mp - 245- 247^{0}C. The Compound gave positive colour reactions with LB reagent (pink), VS reagent (blue), AS reagent (blue-violet) and 20% H_2SO_4 (pink). A solution of **H-3** when treated with LB reagent gave a greenish yellow colour (triterpenoid). Also it gave blue colouration with neutral ferric chloride (phenolic). It decolourised the pink colour of Baeyer's reagent indicating unsaturation in the molecule. The details of the spectra are given as follows:-

IR(KBr) ν_{max} (cm $^{-1}$) : 3361.7 (O-H str), 2929.7, 2856.4 (C-H str-asym & sym),1726.2 (acetoxyl carbonyl str), 1697.2 (α,β-unsaturated ester carbonyl), 1631.7 (-C=C str), 1375.2, 1461.7 (C-H bend), 1251.7 (C-O-C-str).

^{1}HNMR($CDCl_3$)(δ) : 0.905 (H_3-23&30,S), 0.951 (H_3-24&29,S), 0.876 (H_3-25,S), 1.090 (H_3-26,S), 0.858 (H_3-27,S), 0.819 (H_3-28,S),1.253 -1.676 (all -CH_2's & CH's), 2.043 (-OCO-CH_3,S), 3.642 (H-22,t,J=6.6Hz), 4.459 (H-3,dd, J = 6.0,9.6 Hz), 5.532 (H-

15, dd, J = 3, 8.1Hz), 6.836 (H-2H, d, J = 13.2Hz), 5.83 (H-βH,d, J = 12.9Hz), 7.645 (2H-2',6',d, J= 8.4Hz), 6.805 (2H-3',5',d,J=8.1Hz).

^{13}CNMR($CDCl_3$)(δ) : 81.1(C-3),158 (C-14),117 (C-15), 81.1 (C-22), 127 (C-1'), 132.4 (C-2',6'),115(C-3',5'),157 (C-4'),143 (C-α),117 (C-β), 171.1 (-CO_2-), 15.5-55.7(CH,CH_2,CH_3 carbons).

FAB M S (m/z) : 466, 451, 407.391,344,343, 203.

From the above chromatographic and spectroscopic data, the compounds in **H-3** are identified as a mixture of 22- hydroxy taraxeryl acetate and 22-hydroxy taraxeryl - *cis*- p- coumarate.

VI.8. 7,24-Dihydroxy lanosta-22,25-dien-3β -ol-*trans*-p-coumarate (H-4)

The column on elution with petroleum ether-ethyl acetate in the ratio 4:1 gave compound **H-4** as the fourth fraction. It is further purified by preparative TLC and is recrystallized from benzene -chloroform mixture (1:2). It is obtained as pink crystals and melted at 193-195^{0}C. The compound gave positive colour reactions with LB reagent (blue-green), VS reagent (blue), AS reagent (blue-violet) and pink colour with 20% sulphuric acid (steroidal triterpenoid). It also decolourised Baeyer's reagent indicating unsaturation in the molecule. The spectral data are given as follows:

IR (KBr) ν_{max} (cm^{-1}) : 3600-3270 (O-H str), 1707(α,β-unsaturated carbonyl, str), 1676 & 1606 (aromatic C=C str & C-O str), 2937.4 & 2860.2 (C-H str, asym & sym)

^{1}HNMR($CDCl_3$)(δ) : 3.948(H-3,m),3.549(H-7,m),5.41(H-22,d,J=5.7 Hz), 5.352 (H-23,d,J=3.6 Hz), 5.156 (H-25,dd,J=8.4,15.3Hz), 5.015 (H-26,dd,J=8.4,15.3 Hz), 4.58(H-26,dd, J=6,9.3 Hz), 6.3 (H-2',d,J=15.6 Hz), 7.6 (H-3',d, J=15.6 Hz), 6.92 (2H-5'9',f,J=8.4 Hz), 7.43 (2H-6'8', d, J=8.4 Hz), 5.857 (phenolic OH, br), 4.428 (alcoholic OH), 0.914-1.105 (CH_3's), 1.160-1.654 (CH_2's and CH's).

^{13}CHNMR ($CDCl_3$)(δ) : 81 (C-3), 72 (C-7), 121.9 (C-22), 129.4 (C-23), 138.4 (C-25), 117 (C-26), 158(C-1'), 116.3 (C-2'(β)), 141.1 (C-3'(α)), 127.3 (C-4'),130 (C-5',9'), 115.9 (C-6',8'), 157.8 (C-7'),17.6-56 (**C**H,**C**H_2,**C**H_3 carbons).

FABMS (m/z) : 570 (M^+- 2 x 18),555,476,446,432,283,268, 256,230,146,109.

The compound **H-4** thus characterized as 7,24-Dihydroxy lanosta-22,25-dien-3β -ol-*trans*-p-coumarate.

VI.9. 16-hydroxy taraxeryl-*trans*-p-coumarate (H-5)

Elution of the Column with petroleum ether and Ethyl acetate combination in the ratio (3:1) furnished the compound **H-5** as the fifth fraction. It is further purified by preparative TLC. It is recrystallized from acetone to yield a slightly greenish coloured crystals (75mg); mp - 260 - 262^{0}C. The compound gave positive colour reactions with L B reagent (red), V S reagent (blue - black), A S reagent (red -violet) and 20% H_2SO_4 (brown), characteristic of triterpenoid. It decolourised Baeyer's reagent indicating unsaturation in the molecule. It also gave a bluish green colouration with neutral ferric chloride (phenolic group). The spectral data are given as follows:-

IR(KBr) ν_{max} (cm^{-1}) : 3355.9 (O-H, str), 2933.5, 2858.3 (C-H str, asym & sym), 1705 (α,β-unsaturated ester carbonyl, str), 1267 (C-O-C str), 1458.1, 1379.4 (C-H, bend), 1649 (-C=C-, str).

^{1}HNMR ($CDCl_3$) (δ) : 0.914 (H_3 - 23, S), 0.956 (H_3-24,S), 0.981 (H_3-25,S), 1.105 (H_3 - 26,S), 0.914 (H_3-27,S), 0.826 (H_3-28,S), 0.956 (H_3-29,S), 0.914 (H_3-30,S), 4.597 (H-3, m), 5.264 (O-H), 5.544 (H-15,d, J = 6.6 Hz), 6.3 (H-β, d, J = 15.9Hz), 7.602 (H-α,d, J = 15.9Hz), 6.837 (2H -3',5',d, J = 7.8Hz), 7.436 (2H- 2',6',d, J = 8.1Hz). 1.48 - 1.584 (all - CH_2's & CH's).

^{13}CNMR($CDCl_3$) (δ) : 81(C-3), 158 (C-14), 117 (C-15), 69.7(C-16), 128.9 (C-1`), 129.9 (C-2',6'), 115.9 (C-3',5'), 157.5 (C-4'),143.9 (C-α), 116.4 (C-β), 15.6 - 55.7 (**C**H,**C**H_2,**C**H_3 carbons).

FABMS (m/z) : 448, 433, 204, 203, 147.

The Compound **H-5** thus identified as 16-hydroxy taraxeryl-*trans*-p- coumarate.

Section 1

The Phytochemistry of Tabernaemontana species -A review

Section 2

Phytochemical investigation of Tabernaemontana alternifolia

Jose E.A " Phytochemical evaluation of some plants used in folk medicine" Thesis. Department of Chemistry , University of Calicut, 2002

CHAPTER - VII

Section I

THE PHYTOCHEMISTRY OF TABERNAEMONTANA SPECIES - A REVIEW

Tabernaemontana is one of the genera of the family Apocynaceae, sub family plumeroideae and tribe Tabernaemontanoideae. The tribe Tabernaemontanoideae contains, according to Pichon[165], twenty closely related genera. The largest of these are the genera Tabernaemontana with 140 species and Ervatamia with 92-96 species. In some species these genera are synonymous. More than twenty five species of Tabernaemontana occur distributed in different parts of the world[166]. Of these *T. coronaria, T.heyneana, T.sphaerocarpa, T.dichotama* and *T.crispa* are reported to occur in India. In view of the structural correlation's found among the constituents of these genera, it was of interest to investigate other members of the same genera in the hope of finding biogenetically related compounds. Chemical examination of different parts of these plants have been carried out by different workers.

Isolation of several alkaloids, viz., Voacamine, Voacangine, Geissospermine, Coronaridine, Ibogamine, Olivacine and others from various species of Tabernaemontana has been reported[167].

Most of the phytochemical works in various Tabernaemontana species are mostly carried out on leaves, latex, fruits, stem and root bark. The important secondary metabolites isolated from them are as follows. From the Indian varieties of *T. coronaria* several compounds belonging to the class of alkaloids, steroids and triterpenoids have been isolated. The *T. coronaria* has yielded α - amyrin, β - sitosterol and lupeol from stem bark[168] and also a bacteriolytic enzyme from latex[169]. In addition to these voacristine, coronaridine and five other unidentified alkaloids from root bark[170] and α - amyrin, β - sitosterol and two unidentified alkloids from leaves[171] have been isolated from *T. coronaria.* Non Indian varieties of *T. corornaria* are reported to contain alkaloids Tabernaemontanine, Coronaridine, dregamine[172], 19(20) - dehydroervatamine, 2-epi - ervatamine and 20-epi-ervatamine[173] besides several unidentified alkaloids[174]. It is reported that the alkaloid isovoacristine[175] has been identified from *T. laurifolia.*

The chromatography of the alkaloidal fraction obtained by the extraction of the root of *T. psychotrifolia* yielded Coronaridine[172], Voacangine, Voacamine and crystalline yellow alkaloid, olivacine. The

compound olivacine was shown to be isomeric with ellipticine, an anti-tumor alkaloid first obtained from Ochrosia elliptica[176].

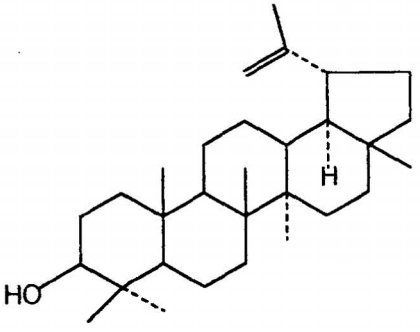
The Chromatography of the alkaloid fraction prepared from the benzene extract of the root of the *T. oppositifolia* yielded several known alkaloids[172]. Elution with benzene gave first crystalline ibogamine, then coronaridine, and finally voacangine. After elution with benzene - chloroform (1:1), voacamine was obtained. It was reported that the alkaloid voacangine and voacamine were obtained from *T. australis*.

Non - Indian varieties of T.heyneana are reported to yield coronaridine and heyneanine besides unidentifeid alkaloids[177,178].

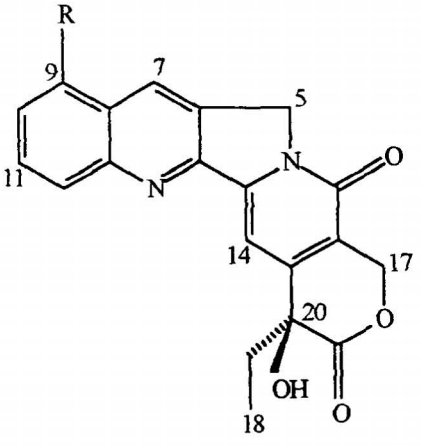
Most of the compounds isolated from the genus Tabernaemontana are known for their cytotoxic and anti tumor activities[179]. The alkaloid olivacine obtained from *T. psychotrifolia* was found to be isomeric with ellipticine. *T. divaricata* (L.). Syn. *E. coronaria* has been used as a cancer remedy in Taiwan[180]. The aerial parts of *T. heyneana* were previously reported[181] as having marginal cytotoxic activity.

Structures of some important compounds reported from

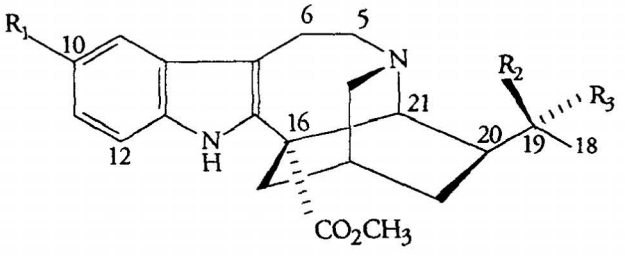
Tabernaemontana alternifolia



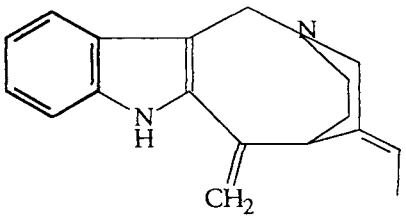
Lupeol



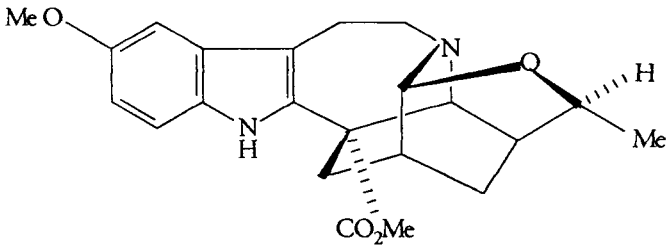
Camptothecin (R = H)
9 - Methoxy camptothecin (R = OCH_3)



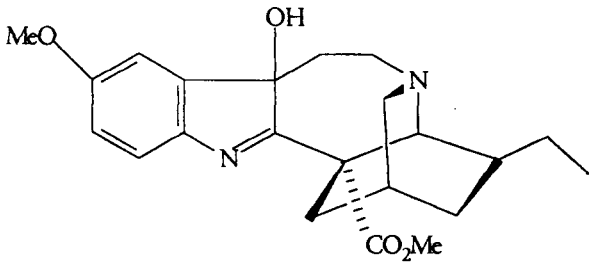
Coronaridine (R_1 = H, R_2 = H, R_3 = H)
Voacangine (R_1 = OCH_3, R_2 = H, R_3 = H)
10-Hydroxy Coronaridine (R_1 = OH, R_2 = H, R_3 = H)
19 S - Voacangarine (R_1 = OCH_3, R_2 = OH, R_3 = H)
Voacryptine (R_1 = OCH_3, R_2R_3 = $=O$
19 S - Heyneanine (R_1 = H, R_2 = OH, R_3 = H)



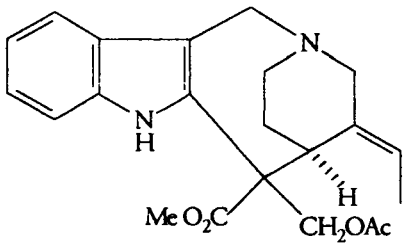
Pericalline



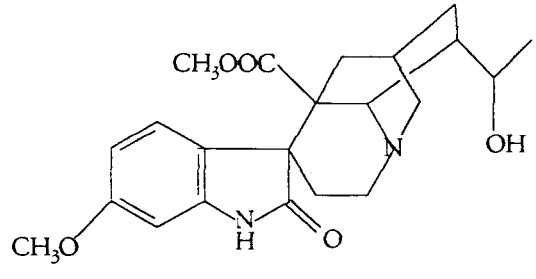
Heyneatine



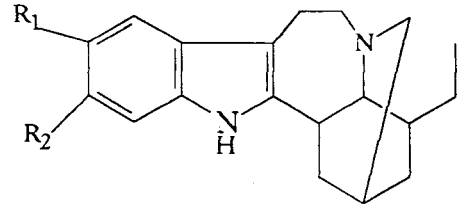
Voacangine hydroxyindolenine



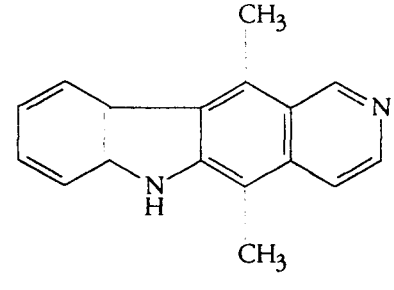
O - Acetyl vallesamine



Tabernoxidine



Ibogamine (R_1 = R_2 = H)
Ibogaine (R1 = OCH_3, R_2 = H)
Tabernanthine (R_1 = H, R_2 = OCH_3)



Ellipticine

Follicle with seeds of *Tabernaemontana alternifolia* Linn.

Section 2

PHYTOCHEMICAL INVESTIGATION OF *TABERNAEMONTANA ALTERNIFOLIA*

VII.2.1. Introduction

Tabernaemontana alternifolia Linn. Syn. Tabernaemontana heyneana Wall., Ervatamia hyneana (Wall.) Cooke, a plant belonging to Apocynaceae family, is widely grown along the coastal region of India. Tabernaemontana with more than 140 species is one of the largest genera of this family. In Malayalam it is known as 'KAMPIPALA'. The extracts of the different parts of the plant are used as Folk and Ayurvedic medicines.

T. alternifolia plants are small trees of 2.4 - 4.5 m height[182]. Their bark is grey and rough. Their leaves are simple, opposite, elliptic - oblong, acuminate, glabrous and prominently nerved; petioles 8 - 20mm long and dilated at the base. Buds are clavate and rounded at the apex. Flowers are in pedunculate and white in Corymbose cymes. Peduncles are of 2.5 - 5 cm long and pedicles are of 1.3 - 2.5 cm long. Calyx is 5mm long, Coriacious and glabrous. The lobes are 2 mm long, broadly ablong, obtuse, with membranous margins. The Corolla tube is of 1.6 - 2.5 cm long, salver shaped, cylindrical and dilated at the top. The stamens are epipetalus (5) and inserted with in the dilated part of corolla tube. The filaments are very short and anthers are linear - acute. The ovary is

bicarpellary, syncarpous, style slender, stigma oblong with a bifid apiculus. Fruits are follicles and becomes Orange - Yellow when matured. It is subsessile, curved, somewhat boat shaped, usually with a short beak which is often recurved and has two sharp side-ribs. Seeds are surrounded by a red pulp and of 8 mm long.

The plant is found distributed in different parts of the world. It is met with in Bengal and South India, especially in Kerala. In Kerala, it is found in Western Ghats in Malabar and Travancore, in open forests.

Tabernaemontana alternifolia has been recognized as a medicinal plant in folk medicine. The flowers are used in inflammation of the Cornea. It is useful in 'Kapha', biliousness and diseases of the blood. The juice of flowers mixed with oil is used to relieve the burning sensation of sore eyes; also used in skin diseases. It is used as a purgative. It is useful in paralysis and lessens pain in the limbs and the joints. Root is used as a local anodyne. Root bark is anthelmintic. Root or root bark is chewed for the relief of tooth ache. Root rubbed with water into a thin paste and administered, acts as vermicide; the same mixed with lime juice is applied to remove opacities of the cornea; also to other eye-diseases. Its charcoal is good for ophthalmia; the oil is good for epilepsy (Yunani). The wood is employed medicinally as a refrigerant. The milky juice is febrifuge and anthelmintic. The milky juice is mixed with oil is rubbed

into the head to cure pain in the eyes. Milk juice of leaves is dropped into the eye to cure ophthalmia; also a cooling application to irritable surfaces; to wounds to prevent inflammation[182].

VII.2.2. Previous studies on *T. alternifolia*

T. alternifolia of Apocynaceae is reported to be a medicinally important plant. Many of the compounds isolated from leaves, latex, fruits, stem and root bark of the plant are found to be biologically active. This plant is a source of many alkaloids which have been studied by pharmacologists as potential stimulants. Some of them displayed both cytotoxic and antitumor activities. The important secondary metabolites isolated from this plant are as follows: *T. alternifolia* yielded lupeol and other triterpenoids from bark[183]; unidentified triterpenoids from fruits[184]; heyneanine from roots and bark[185]; Coronaridine from seeds[186,187] and roots. The coronaridine and heyneanine alkaloids displayed cytotoxic activity.

The alkaloid isovoacristine, a rare alkaloid, has been identified from the leaves of *T. alternifolia*. This compound is reported to have both cholinergic and antihistaminic activities[175].

From the leaves of the plant a minor alkaloid designated as tabernoxidine was isolated [188].

The wood and stem bark of this plant yielded fourteen indole alkaloids and three triterpenoids[189]. Some of these isolates displayed cytotoxic activity. The isolated alkaloids are voacangine, Coronaridine, Voacangine hydroxyindolenine, Voacryptine, 19S - Heyneanine, 19S - Voacangarine, 10-Methoxyeglandine-N-oxide, 10-Hydroxycoronaridene, Heyneatine, O-Acetyl vallesamine, Pericalline, Dihydrocondylocarpine, Camptothecin and 9-Methoxy camptothecin. The alkaloids Camptothecin and 9 - Methoxy camptothecin are known for their potent antileukemic and antitomor properties. The alkaloid Camptothecin also inhibits herpes and other mammalian viruses[190,191].

VII.2.3. Present Work

The phytochemical examinations so far conducted in *T. alternifolia* have revealed that this plant is a potential candidate for further phytochemical study.

Most of the phytochemical works conducted on this plant are concentrated on flowers, leaves, root and stem bark. Studies on the fleshy red pulp that surrounds the seeds are not yet been carried out. In view of the chemical as well as the pharmacological importance of the compounds isolated form this plant, it was of interest to investigate further the same plant in the hope of finding new Carotenoids and /or flavonoids. So the present work is undertaken to isolate the compounds

from the red pulp that surrounds the seeds and to characterize them by chromatographic and spectroscopic methods.

VII.2.4. Results and Discussion

The pulpy red material around the seed of Tabernaemontana *alternifolia* is taken in a round bottomed flask. It was extracted with benzene by keeping it for 3 days in the dark at laboratory temperature. The combined extract was then concentrated under reduced pressure using rotary flash vacuum evaporator at a temperature below 40^0C. The extract was then adsorbed on silica gel and packed in a column. The column was then eluted with petroleum ether and petroleum ether - ethylacetate mixture of varying composition. Two different compounds obtained from the chromatographic purification are designated as **T-1** and **T-2** . The details are given in **Table 7.1.**

Table 7-1

Compounds isolated from the benzene extract of red pulp that surrounds the seed of T. *alternifolia*

Compounds	Eluant Composition	Melting point
T-1	Petroleum ether	38 - 40^0C
T-2	Petroleum ether - Ethylacetate (9:1)	125 - 127^0C

Thin layer chromatographic characterisation of compounds **T-1** and **T-2** is carried out using various spray reagents. The results are given in **Table 7.2.**

Table 7.2

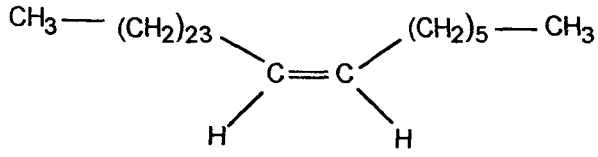
TLC Characterization of compounds T-1 & T-2 isolated from *T. alternifolia* using spray reagents

Compounds	Without spray reagent	Borntrager reagent	Vanillin- sulphuric acid reagent	Baeyer's reagent	20% H_2SO_4	UV	Class of compound
T-1	Not visible	---	Brown	Decolourised	Black	---	Olefine
T-2	Yellow	Deep yellow	Brown	Decolourised	Reddish - brown	Highly flourescent	May be carotenoids

VII.2.5. Characterisation of T-1 (*Cis*- Dotriacont -7-ene)

The compound **T-1** was obtained as the first fraction on elution of column with petroleum ether. The compound was washed with cyclohexane several times and recrystallized from n-hexane to yield a colourless waxy substance which melted at 38-40^{0}C. This compound did not answer Vanillin-sulphuric acid reagent and Borntrager (alcoholic KOH) colour reactions indicating that it was not a terpenoid or a

flavonoid. It decolourised pink colour of Baeyer's reagent indicating that it is an unsaturated molecule. It gave a single spot on TLC analysis. The detailed IR, ^{1}Hnmr, ^{13}Cnmr and mass spectral data investigations identified the compound as *cis*-Dotriacont-7-ene. The molecular formula was determined to be $C_{32}H_{64}$ by FABMS. The structure of the compound is identified as **T-1** based on the data obtained on the above spectroscopic studies.



T-1

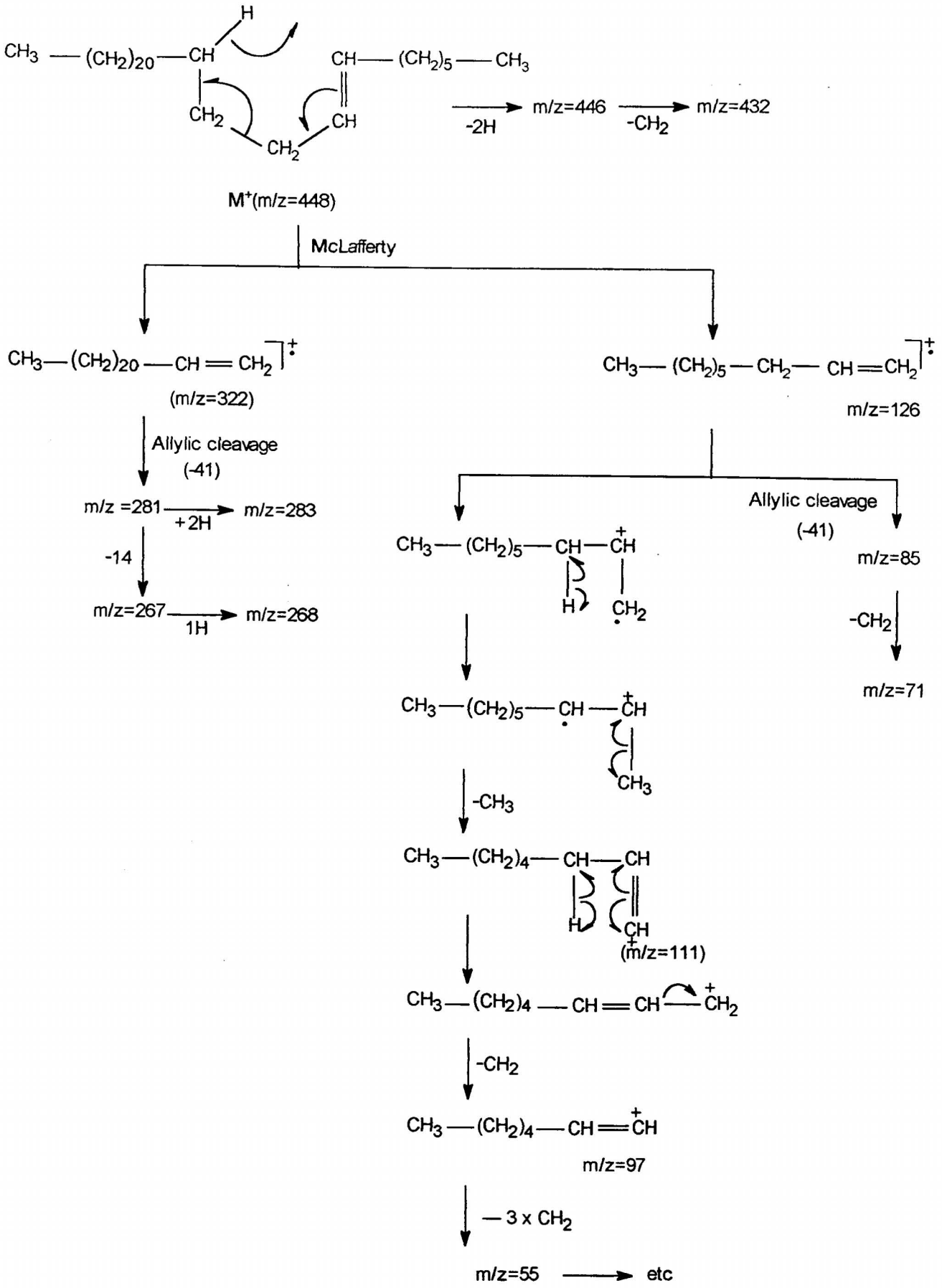
The IR spectrum gave an absorption at 1637.5 cm^{-1} which was attributed to the presence of a C = C double bond. It also showed bands at 2920 cm^{-1} and 2850.6 cm^{-1} which was due to C-H stretching of CH_2 (asym & sym), and bands at 1463.9 and 1377.1cm^{-1} showing C-H bending (aliphatic). Other bands seen in the spectrum at 3388.7cm^{-1} and others are taken as due to the presence of moisture and can be ignored.

The ^{1}Hnmr and ^{13}Cnmr spectral data also agree very well with the suggested structure for the compound **T-1**. The ^{1}H signal at 5.347 ppm (2H, t, J = 4.8 Hz) could be attributed to olefinic protons. The coupling constant value indicates that the olefinic protons are *cis* to each other and

correspondingly the compound **T-1** is a *cis*-olefine. The ^{1}H signal at 2.004 ppm is ascribed to CH_2 groups next to the double bond. The PMR chemical shifts at δ_H1.256 - 1.708 were assigned for all other CH_2 groups than those next to double bond. The other signals at δ0.857 - 0.901 could be due to the two overlapping triplets of the two -CH_3 groups. The only disagreeing factor is that the integration of the olefinic protons seems to be only one. This may be due to the identical nature of the olefinic H atoms. This is supported by the ^{13}C and ^{13}C-DEPT spectral data. The ^{13}C chemical shifts from δc 14.1 to 37.5 could be assigned to the carbon atoms of CH_3 and CH_2 carbons in the molecule. Also the ^{13}C signal at 129.9 ppm could be ascribed to the olefinic C atoms.

In its mass spectrum the molecular in (M^+) appeared at m/z 448. The other peaks obtained at m/z values 446, 432,419, 283, 268,97, 85, 71, 55 etc. are all in accordance with the proposed structure for **T-1**. The possible structures for the selected ions in the mass spectrum of the compound **T-1** can be depicted as given in **scheme 7-1.**

The sequential loss of the mass units of 14 is characteristic of mono alkenes. The formation of the peaks at 283,268,97....can also be explained by an allylic cleavage of molecular ion. This is in confirmation of the position of the double bond at 7.


M+(m/z=448)
-2H
m/z=446
-CH2
m/z=432
McLafferty
(m/z=322)
Allylic cleavage
(-41)
m/z =281
+2H
m/z=283
-14
m/z=267
1H
m/z=268
m/z=126
Allylic cleavage
(-41)
m/z=85
-CH2
m/z=71
-CH3
(m/z=111)
-CH2
m/z=97
— 3 x CH2
m/z=55
etc

Scheme:7.1

Consideration of IR, ^{1}H -& ^{13}C -NMR and Mass spectral data permitted the complete the structure of the compound to be assigned as *cis*-Dotriacont-7-ene.

VII.2.6. Characterization of Compound (T-2)

The compound **T-2** was obtained as the second fraction from column chromatographic separation of the extract of red pulpy seed coating of *T. alternifolia*. It was recrystallized from benzene-ethyl acetate in the ratio 1:1 as yellow powder. It melted at 125-127^{0}C. Thin layer chromatographic characterization of the compound using spray reagents indicated that it may be a carotenoid.

IR spectrum gave a strong absorption at 1716.5 cm^{-1} indicating the presence of a carbonyl group. The absorption between 3700-3415.7cm^{-1} indicates the presence of a hydroxyl group in the compound. It also showed bands at 2931.6 cm^{-1} and 2977.9 cm^{-1} which was due to C-H str (asym & sym) and bands at 1380.9 cm^{-1} and 1450.4 cm^{-1} were due to C-H bending. From these informations it could be understood that the compound may be a hydroxyl group containing carbonyl compound, most probably an ester or acid. The ^{1}H- and ^{13}C-NMR spectral data obtained were not much informative to characterize the compound. In the Mass spectrum the peak with highest m/z value is seen at m/z 448, most probably the molecular ion. Other peaks in the spectrum were not much

supporting to propose a structure for **T-2**. So the compound has been again sent for spectral analysis and the results are awaited.

VII.2.7. EXPERIMENTAL

Instrumentation

The melting point of compounds isolated were determined using Toshniwal Capillary melting point apparatus and are uncorrected. The IR spectra of the compounds were recorded using a SHIMADZU FTIR - 8101 - A spectrometer in KBr pellets. The ^{1}H - and ^{13}C - NMR spectra were obtained on a VARIAN UNITY PLUS - 300 spectrometer with tetra methylsilane (TMS) as internal standard using $CDCl_3$ as solvent. The chemical shifts are recorded in δ(ppm) values. Multiplicities of ^{13}C - NMR signals were determined by means of the DEPT method. FABMS were obtained using a JOEL SX-102(FAB) mass spectrometer.

Column chromatography was performed on Silica gel (Acme, 100 - 200 mash). The columns were prepared as a slurry with suitable solvents and eluted with selected chromatographic solvents. Thin Layer chromatographic analysis was performed on TLC grade silica gel - G (Acme). All solvent used in the course of the work were purified by standard procedures described by 'Vogel'.

Purity of the compounds was checked by TLC. The spots were visualised by UV irradiation and by spraying with Borntrager reagent (alcoholic potassium hydroxide). Vanillin - sulphuric acid, 20% sulphuric acid and Baeyer's reagent. Borntrager reagent is 10% ethanolic Potassium hydroxie solution . The other spray reagents used are prepared as in section 6.2.

Plant material

The pulpy red material that surrounds the seeds of *T. alternifolia* was collected from the University Campus of Calicut University, Kerala and identified in the Department of Botany, Calicut University by Dr.A.K.Pradeep. A voucher specimen (No: 70561) has been deposited in the Herbarium [CALI] of Department of Botany, University of Calicut.

Extraction and isolation

The pulpy red material around the seeds of *T. alternifolia* (1kg) taken in a R.B.flask was extracted with benzene at room temperature and kept for 3 days in the darkness. This precaution is taken in order to prevent photoisomeriation and photo-destruction of Carotenoids that may present in the extract. The extract was then concentrated under reduced pressure to about 300 ml using rotary vacuum flash evaporator. This extract was then adsorbed on Silica gel (100 - 200 mesh) and packed

above a column of silica gel (500 gm) in a glass column (3 cm X 100 cm). The column was then eluted with petroleum ether and petroleum ether - ethyl acetate mixture of varying compositions. 10 ml aliquots were collected in test tubes and the contents of each test tube was compared by thin layer chromatography. The fractions which gave identical spots were pooled. These fractions were grouped according to their homogeneity judged from the TLC analysis. The petroleum ether - ethyl acetate mixture fractions were combined and subjected further to column chromatography on Silica gel (100 - 200 mesh) and eluted with solvents of increasing polarity viz petroleum ether, petroleum ether - ethyl acetate and ethyl acetate in that order. Several 10 ml fractions were collected and each fraction was checked by TLC. Identical portions were pooled together and the solvent was evaporated under reduced pressure. Different compounds obtained from benzene extract of seed coatings of *T. alternifolia* were designated as **T-1** and **T-2**. The compound **T-1** was recrystallized from n - hexane to yield a colourless substance (120 mg) which melted at 38 - 40^0c. The compound **T-2** was recrystallized from benzene - ethyl acetate (1:1) as yellow powder (90mg) which melted at 125 - 127^0C.

VII.2.8. *Cis*-Dotriacont - 7- ene (T-1)

Elution of the column with petroleum ether yielded colourless substance of compound **T-1**. It is recrystallized from n - hexane to get colourless waxy substance (120mg); mp - 38 - 40^{0}C. It did not answer any characteristic colour reactions with spray reagents except for Baeyer's reagent. It decolourised Baeyer's reagent indicating unsaturation in the molecule. The spectral details are as follows:-

IR (KBr) ν_{max} cm^{-1} : 2920, 2850.6 (C - H Str, asym & sym), 1637.5 (C = C str), 1463.9, 1377.1 (C-H bend).

^{1}HNMR($CDCl_3$)(δ) : 0.857-0.901 (CH_3 groups), 1.256-1.708 (CH_2groups), 2.004 (CH_2 groups next to the double bond), 5.347(2H,t,J=4.8 Hz, olefinic protons)

^{13}C NMR($CDCl_3$) (δ) : 14.1 to 37.5(CH_2 and CH_3 carbons), 129.9 (olefinic carbons)

FABMS (m/z) : M^+(448), 446,432, 419, 283, 268, 97, 85, 71, 55, etc.

SUMMARY

Jose E.A " Phytochemical evaluation of some plants used in folk medicine"
Thesis. Department of Chemistry , University of Calicut, 2002

SUMMARY

Plants have fed the world and cured the ailments of its population since time immemorial. The study of naturally occurring substances having medicinal and other useful properties has been a subject of rapid development and an interesting field of active research. Compounds isolated from natural sources continue to occupy an important place among useful products of modern medicine. The systematic study of medicinal plants used in traditional medicine and the characterisation of compounds isolated using modern scientific tools have increased the economic importance of the plant pharmaceuticals. By and large the pharmacological activity of a medicinal plant resides in the so called secondary metabolites like terpenoids, steroids, alkaloids and the other classes of chemical entities.

The present study is aimed to identify the active compounds present in the leaves of ***Hibiscus schizopetalus*** of Malvaceae family and in the pulpy red coating around the seeds of ***Tabernaemontana alternifolia*** of Apocynaceae family. These plants have been recognized as medicinal plants in folk medicine and so these form an ingredient in many of the folk and Ayurvedic preparations.

The Phytochemical investigation of petroleum ether extract of leaves of *H. schizopetalus* resulted in the isolation and characterisation of five compounds, of which four were identified and found to be new compounds. The compounds are Cis-Hentriacont-3-ene, 22-Hydroxytaraxeryl acetate, 22-Hydroxytaraxeryl- cis-p-coumarate, 16-Hydroxytaraxeryl-trans-p-coumarate and 7,24-Dihydroxylanosta-22,25-dien-3β-ol-trans-p-coumarate. All compounds except *cis*-Hentriacont-3-ene are triterpenoids and not reported earlier.

The study of the secondary metabolites from *H.schizopetalus* is further extended with the methanolic extract and the characterization of the compounds obtained are under investigation.

The phytochemical examination of benzene extract of red pulpy material that surrounds the seeds of *T. alternifolia* yielded two compounds of which only one could be characterised. The spectral data obtained for the second compound were not much informative and hence could not be identified. Hence fresh purified sample of compound **T-2** has been again sent for spectral analysis and results are awaited. The first compound is identified as *cis*-Dotriacont - 7- ene.

Structure of all compounds isolated were established with the help of IR, ^{1}H-&^{13}C/DEPT-NMR and Mass spectral data.

However, the structure suggested for **H-4** (7,24-Dihydroxylanosta-22,25-dien-3β-ol-*trans*-p-coumarate) is tentative. To have a conclusive evidence a correlation spectral studies are required. Thus the sample has been sent for HMBC and COSY spectra to RSIC., I.I.T., Bombay and the results are awaited. Due to the lack of time, with the available evidences structure of compound **H-4** has been suggested and the discussion presented.

It is reported that taraxeryl and lanosteryl esters are found to exhibit a variety of useful biological activities. They are known for their anti-allergic, anti-cancerous, anti-hypertensive and anti-inflammatory activities. Certainly, the new compounds from *H.schizopetalus,* being the esters of hydroxy taraxerol and lanosterol, also may possess useful biological properties. However, this has to be ascertained by pharmacological screening technique in tune with its folk medicinal use.

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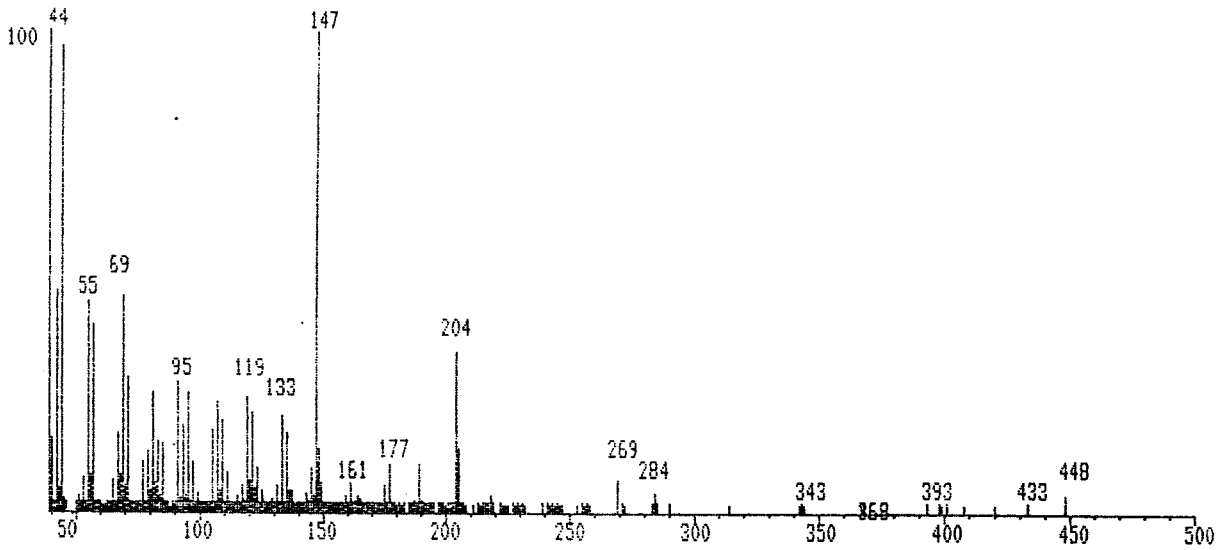

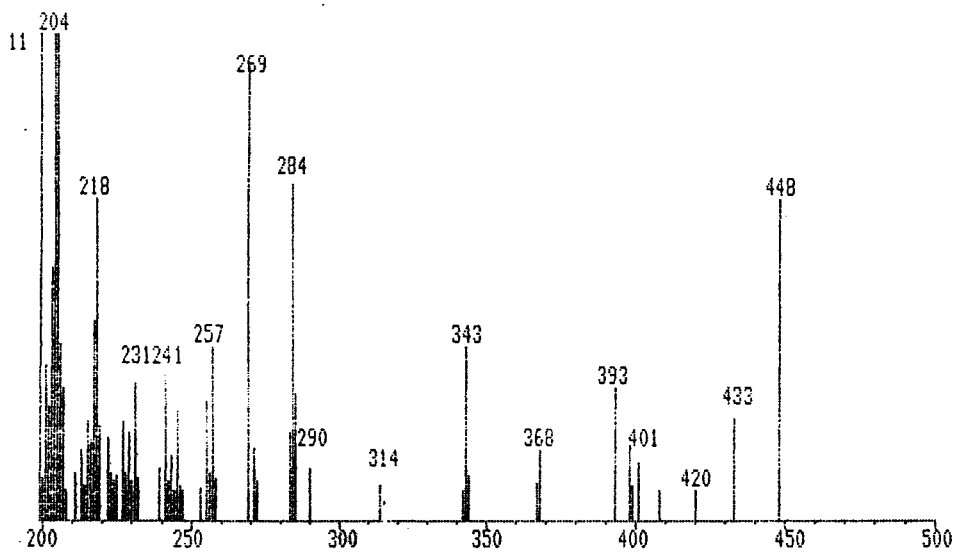

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