CHEMICAL INVESTIGATION ON VITIS QUADRANGULARIS

A Dissertation Submitted in the Partial Fulfilment for the Degree of Master of Philosophy (M. Phil) in Chemistry.

Submitted by
Monimanjusha Mazumder

Roll No. 930309 F
Registration No. 93709
Session : 1992 - 93 - 94.

Bangladesh University of
Engineering & Technology.
July, 2001

Research Laboratory
Department of Chemistry.

TO

MY

BELOVED PARENTS

&

DAUGHTER

রসায়ন বিভাগ
বাংলাদেশ প্রকৌশল বিশ্ববিদ্যালয়, ঢাকা-১০০০

Department of Chemistry
Bangladesh University of
Engineering and Technology (BUET)
Dhaka-1000, Bangladesh.

THESIS ACCEPTANCE LETTER

We hereby recommend thesis entitled Chemical investigation on "*Vitis quadrangularis* Wall. (Harzora Lata)", Presented by Monimanjusha Mazumder, Roll No. 930309 F, Registration No. 93709, Session 1992-93-94 to accept as partial fulfilment of the requirements for the degree of Master of Philosophy (M. Phil) on 3rd July, 2001.

Board of Examiners :

1. Dr. Nazrul Islam
   Associate Professor,
   Chemistry Department, BUET,
   Dhaka, (Supervisor).
   — Chairman 3.7.2001

2. Prof. Dr. Enamul Huq.
   Department of Chemistry
   BUET, Dhaka.
   — Member ৩/০৭/২০০১

3. Prof. Dr. A. K. M. Matior Rahman
   Head, Department of Chemistry
   BUET, Dhaka.
   — Member 03/07/2001

4. Prof. Dr. Md. Abdul Quader
   Department of Chamistry,
   Dhaka University, Dhaka.
   — Member (External) 03.07.01

03/07/2001

(Dr. A. K. M. Matior Rahman)
Professor & Head
Department of Chemistry
BUET, Dhaka-1000.

-el : 9665614, **PABX:** 866833-38, 864640-4, 868344-49 Extn 7335 **Fax :** +880-2-863026, +880-2-863046 **e-mail:** buetlib@bd.drik.net

CANDIDATE'S DECLARATION

It is hereby declared that this thesis or any part of it has not been submitted elsewhere for the award of any degree or diploma.

(Monimanjusha Mazumder)
Name of the Candidate

ACKNOWLEDGEMENT

I am highly pleased to express my best regards, profound gratitude, indebtedness and deep appreciation to my research supervisor and reverend teacher Dr. Nazrul Islam, Associate prof. Department of Chemistry, Bangladesh University of Engineering and Technology, for his valuable and constant supervision, inspiring guidance, enthusiastic encouragement, sagacious advice and an affectionate surveillance through the entire period of my research work.

I am greateful to my reverend teacher Dr. A.K.M Matior Rahman, Professor and Head of the Department of Chemistry, BUET, Dhaka. For his valuable suggestion during the course of my study.

I would like to express my deepest sense of gratitude to all my teachers of the Chemistry Departmet of BUET, Dhaka for their constant encouragement and help during this work.

I would like to offer my thanks to my friend Md. Mosaddek Ahmmed, Rubina Apa and Akram Hossain for their constant help.

I also offer my best regards to Dr. Nasim Sultana and Dr. Mozaffar of BCSIR for recording all NMR and GC-MS spectra and Mr. Akram Hossain for recording IR spectra.

I an graliful to Prof. Dr. M. Ashraf Alam of Bristol University, UK for helping me to carryout a detailed review work for this thesis.

I really a lot realy owe to my husband, daughter and all family members for their constant encouragement to achieve the Master of Philosophy degree.

I am also very much greateful to all the staffs of the Chemistry Department, BUET, for their co-operation and encouragement during my research work, specially Mr. Mamun for his constant company.

CONTENTS

CHAPTER - 1

INTRODUCTION

CHAPTER - 2

EXPERIMENTAL

CHAPTER - 3

RUSULTS AND DISCUSSION

3. RUSULTS AND DISCUSSION

LIST OF TABLES

LIST OF TABLES

CHAPTER - 1

1.INTRODUCTION

1.1 GENERAL

Nature in one sense is compounded of a series of interdependent cyclic systems, the carbon, nitrogen, phosphorous and as many other cycles as there are elements utilized by plants and animals. Each of these represent, one facet of the interdependence of things "natural". The study of natural products has always been the primitive fields of investigation of the discipline of chemistry in every country of the globe. Man's dependence on plants for the essentials of his existence has been of paramount importance in his life, since human race began. Civilization however, has brought with an ever-increasing man's requirements supplied in great part by plants. Thus study of plant as an organized science started and flourished from man's necessity for plants as producers of raw materials of four basic needs- food, cloth, shelter and medicine. The Plants very seldom use these products by themselves but serve in many ways and are called the secondary metabolites or natural products. The term **"natural products"** is recognized by the chemists as meaning those secondary metabolites, usually of relatively complex structure, which are of more restricted distribution and more characteristic of specific botanical sources than are the compounds produced by primary metabolic processes. It may be noted that the secondary plant products aren't "function less anomalies" or simply the "end products" of metabolism.A general characteristic of "natural products" is that few of them have a clearly recognized function in the metabolic activities of the organisms in which they are found. Although much is known about the chemical nature of most of these compounds, still some unknown one's remain to be isolated and characterized. Some known compounds are in general term carbohydrates, alkaloids, essential oils, terpenes, steroids, vitamins, color pigments etc. Some of the natural products are of vital importance to the functioning of the species- producing them while others appeared to have no function at all. On the basis of chracteristics of the natural products, the plants are classified into the following groups :

1. Harmful or Poisonous plants
2. Useful or Medicinal plants.

Plants, which serve us as sources of energy and effective drugs, for example, the natural products such as vitamins, hormones, antibiotics, analgesic extracted from plants are very important for their medicinal and economical uses.

The importance of natural products and their applications in certain sphere of human life have attracted the attention of chemists and druggists. As a result in the early decades of the civilization, natural products chemistry achieved a revolutionary advancement leading to systematization action of chemotherapy and this fruitful discovery has accelerated the research work for newer medicine. From time immemorial various traditional systems of treatment of diseases like Kaviraze, Ayurvedic and Unani have developed in the world specially in the Indian sub-continent based on the use of the medicinal plants. These medicinal plants were then the only sources to alleviate the suffering of body and mind. Even today these systems of treatment are fairly common amongst the village people of the Indian sub-continent. Foom ancient times the roots of an indigenous plant, *Rauwolfia serpentine* (Beng. Sarpa gandha) has been widely used as an antidote to insect and snake bites, as a febrifuge, as a stimulant to uterine contraction and as a sedative. The tincture made from *Ephedra vulagris*, and *Vitis quadrangularis*[21] Wall. (Beng. Harzora lata) is effective in the treatment of asthma, cardiac failure etc. Every part of the plant *Azadhirachta indica* (Beng. Neem) is reported to have medicinal properties.

Numerous naturally occuring compounds were known by the end of the first quarter of the nineteenth century. In the eighteenth century,the use of the extracts of digitalis and cinchona as cures of certain diseases were very popular and attractive. It was again the medicinal importance of the bile acids, that led to the isolation of animal products cholesterol by Poulletier in 1780. At the beginning of the nineteenth century, when the modern chemistry and pharmacy began to develop, the original impetus to the study of natural product chemistry utilizing medicinal plants received a good boost. Morphine, the hypotic and anaesthetic principle of opium was one of the early (1805)active principle isolated from plant bodies. Quinine form Cinchona bark used in the treatment of malaria atropine from *Atropha belladonna,* cocaine from coca leaves, nicotine from tobacco leaves were other examples. Subsequently a whole series of plants were investigated to detect and isolate the active principles. This led to the discovery of a group of nitrogenous bases having complicated structures and physiological activities and were called alkaloids. The alkaloids of Rauwolfia group such as Reserpine is used in medicine in conditions, including tension and anxiety as well as in the treatment of hypertension. The alkaloid vinblastine from Vinca rosea Linn. has been subject of medical studies particularly in the field of cancer.

In the field of natural product chemistry, modern chemotherapeutic drugs have progressed research work which may lead to the discovery of newer drugs or drugs having, minimum or no side effect. 170 drugs from different plants which are or once were official in U.S.P were used by the North American, Indian. In 1967, 25% or 824 out of 3354 of the trade name or generic name products appeared in 1.05 billion prescriptions filled in the United States,contained one or more ingredients derived from higher plants[1]. In many countries of the world, native medicinal plants are thus looked upon as possible additions to the WHO list of "essential drugs", once their value had been clinically proven[2] .

The people of developing countries like ours, can not afford to use expensive modern chemotherapeutic drugs due to economic problems. We have to remember that traditional medicines are cheap and readily available whereas, the modern medicines with all its glories getting more developed and expensive, thus can not satisfy the necessity of about eighty percent of our people.

Our country abounds with a vast majority of medicinal plants and herbs. The availability of medicinal plants demands the isolation, purification and characterization of physiologically active principles which are actually useful for the treatment of diseases. The study on these plants in our country unfortunately has not been so far very systematic.

A brief list of some of the plants used by traditional practitioners of the sub-continent are given below:

a. Anaemia

The flower of *Vitis vinifera*[21] (Beng. Angurphal) is used for enrichment blood. The dried tuber of *Vitis adnata*[21] is used by the country people as blood purifier. The roots of *Ipomoea turpethum*[3] (Beng. Dud kalmi),theleaves of *Hygrophila auriculata*[4] (Beng. Kulekeshara, Talmakhna), the flowers of *Gmelina arborea* (Beng. Gamari). the fruits of *Phyllanthus embkica*[4] (Beng. Amla,Amladi), the seeds of *Trigonella foenumgraecum*[5] (Beng.khoyer),*Termialia chevula*[6] (Beng. Thunkuni). etc. are used for the remedy of anaemia.

b. Antidiabetes

The roots and leaves of *Coccinia indica*[6] (Beng. Telakucha), are reported to have sugar lowering activity and clinical tests on the capsule made of it have proved to be so.[7] The barks of *Eugenia jamos*.[6] *Momordica charantia*[8] (Beng. Karulla, Usta). the seeds of *Trigonella foenumgraecum*[9] (Beng.Methi), the leaves, seeds and bards of *Mangifera inidca* Linn,[6] (Beng.Aam), *Michelia champaca* Linn[6] (Beng. Kola) etc. are used for the treatment of diabetes.

c. Antifertility

The plants of *Acacia catechu*[10] (Beng. Khoyer), *Abrus precatiorus* Linn.[11] (Beng. Kunch). *Areca catechu* Linn.[12] (Beng. Supari, Shupari), *Carina papaya*[12] (Beng.Papaya, Pipay), the roots of *Plumvago zeylanica* Linn.[13] (Beng. Babla)etc. ate reported to have antifertility activity.

d. Antiseptic

The rongas of East Africa apply the pounded stem of *Vitis quadrangularis*[21] Wall. (Beng. Harzora lata) to wounds.
The leaves of *Cynodon dectylon* (Beng. Durba, Dubla ghash)[4], bulbs of *Allium sativum*[4] (Beng.Rasun), leaves of *Pistia stratiotes* L[4]. (Beng. Topapana). *Azadirachta indica*[3] (Beng. Neem), *Eucalyptus globules*[6], seeds of *Cleome viscosa* L[4] (Beng. hurhura), *Trigonella foenumgraecum*[15] (Beng.'Methi), the plants like *Oxalis corniculata* L[4]. (Beng. Amrul), *Sacchrum officinarum* L[4] (Beng. Akh) etc.are used by people as antiseptic for cuts and wounds.

e. Asthma

The stem beaten into a paste of *Vitis quadrangularis*[21] Wall. (Beng. Harzora lata).
The fruits of *Vitis vinifera* Linn (Beng. Angurphal)[21]
The barks of *Alstonia scholaris*[6] (Beng. Chhatim), *Caesalpinia cristia*[6] (Beng. Nata, Nata koromza), *Eugenia jambolana*[6] (Beng. Jam, Kalajam), *Eugienia jambos*[6] (Beng. Jamrul), the fruits of *Coccinia indica*[6] (Beng. Telakucha), *Mimusops elengi*[6] (Beng. Bokul, Bakul), *Terminalia chebula*[6] (Beng. Horitoki), the leaves of *Datura metal* Linn.[6] (Beng. Dhutora, Dhutara), *Datura stamonium* Linn.[6] (Beng. Shada Dhutara), *Ricinus communis* Linn.[6] (Beng. Verenda), the barks, fruits and leaves of *Aegel marmelos*[6] (Beng. Bel), *Mangifera indica*[6] (Beng. Aam), the

fruits and leaves of *Adhatoda vasica*[6] (Beng. Bashok), the barks and leaves of *Calotropis gigantea*[6] (Beng.Akondo), the plants of *Hydrocotyl asiatica* Linn.[6] (Beng. Thunkuni) etc. are used as medicines for the treatment of asthma.

f. Bronchitis

The roots of *Vitis indica*[21] Wall (Beng. Amdhauka, Amoluka). The flowers of *Vitis vinifera* is used for remedy of chronic bronchitis.[21] The barks of *Acacia farnesiana*[6] (Beng. Khoyer). *Eugenia jambos*[6] (Beng. Jamrul), *Eugenia jambolana* (Beng. Chotojam, jam, kalajam), the leaves and barks of *Acacia arabica*[6] (Beng.Babla), the barks and seeds of *Punica granantum*[6] (Beng.Dalimgach), the leaves of *Psidium guyava*[6] (Beng. Peyara, Piyara), the plants of *Hydrocotyl asiatica*[6] (Beng. Thunkuni),*Vernonia cinerea*[6] (Beng. Kalajira), the leaves and roots of *Lawsonia inermis*[6] (Beng. Mehidi, Mendi) etc, are used for the cure of bronchitis.

g. Cancer

The alkaloids, vincristine and vinblastine isolated from *Vinca rosea*[4] (Beng. Nayantara) are being used against leukemia. The lates of *Ficus raccemora*[4] (Beng. Jagadumur) is useful as anticanceral agent. The leaves of *Rginacanthus nasuta*[4] (Beng. Juipana) are applied in the treatment oif cancer. the plant *Vitex trifolia*[4] (Beng. Panisamalu) shows anticancer activity.

h. Diarrhoea

The leaves and young shoots of *Vitis quadrangularis*[21] (Beng. Harzoralata).The leaves of *Vitis vinifera*[21] (Beng. Angurphal).The bark of *Alstonia scholaris*[4] (Beng. Chhatim) is a valuable remedy in chronic diarrhoea. The bark and seeds of *Albizzia lebbech*[4] (Beng. Pathrkuch) *Ipomoea batatas*[4] (Beng. Mistialu), the plants of *Cynodon dactyoln*[4] (Beng. Durba), *Oalis corniculata*[16] Linn. (Beng. Amrul), the leaves of *Hydrocotyl asiatica*[7] (Beng. Thunkuni) etc. are reported to be effective against diarrhoea.

i. Diuretic

The fruits of *Vitis vinifera*[21] Linn. (Beng. Angurphal). The roots of *Berginia ligulata*[6] (Beng. Pathorchuri, Pathorchuchi), *Lawsonia inermis*[6] (Beng. Mehidi, Mendi), the barks of *Terminalia arjuna*[6] (Beng. Arjun, Arjuna), the fruits of

Eugenia jambolana[6] (Beng. Chotojam, Jam, Kalajam), *Luffa aegytiaca*[6] (Beng.Dhundul), the seeds of *Helianthus annus*[6] (Beng. Surjamoki), *Trigonella* foenumgraecum[17] (Beng. Methi), the leaves and plans of *Heliotropium inducum* Linn.[6] (Beng. Hatirshoor, Hatishoor), the roots and fruits of *Abutilon indicum*[6] (Beng. Pottri, Jhumko), the roots and leaves of *Asparagus racemosus*[6] (Beng. Shotomuli) etc are used as diuretic agents.

j. Dysentry

The barks and seeds of *Acacia catechu*[6] (Beng. Khoyer), *Aegel marmelos*[6] (Beng. Bel), *Diospyros embryopteris* (Beng. Gub), *Mangifera indica*[6] Linn. (Beng. Aam), *Phyllanthus emblica*[6] Linn (Beng. Amloki), the plants and leaves of *Oxalis corniculata*[4] Linn.(Beng. Amrul), *Andrographis Paniculata*[6] (Beng. Kalomegh) etc.are used as medicine for the treatment of dysentry.

k. Gonorrhoea

The leaves of *Acacia farnesiana*[6] (Beng. Guya babla), *Acacia arabica*[6] (Beng. Telakucha), the toots of *Lawsonia inermis*[6] (Beng. Mehidi, Mendi), *Ipomoea kigitata*[3] (Beng. Bhuikumra), the leaves and young shoots of *Heliotropium indicum* Linn[18,19] (Beng. Hatirshor, hatishoor), the roots, barks and leaves of *Abroma augusta* Linn,[6] (Beng. Ulat kombal), the seeds and unripe fruits of *Abelmoschus esculentus*[6] (Beng. Dharosh) etc. are reported tobe helpful or the treatment of gonorrhoea.

l. Hepatitis

Croton oblogifoliur Roxb.[4] (Beng. Chuka, Putri, bragachi) is externally applied to the hepatitic region in chronic hepatitis.

m. Hypertension

The roots of *Rauwolfia serpentina*[20] (Beng. Sarpagandha) are known to be an impotant source of hypertensive and tranquillizer reserpine. Recent clinical trials on the capsule made from the dried powdered leaves of *Moringa oleifera*[7] (Beng. Sajna) have shown encouraging results as anthypertensive.

n. Jaundice

The raisins of *Vitis vinifera* (Beng. Angurphal)[21]. The flowers of *Coccinia indica*[6] (Beng. Talakucha), the leaves and bards of Lawsonia inermis[6] (Beng. Mehidi), the roots of Ipomoea turpethum[3] (Beng. Dud kalmi), the plants of *Sphaeranthus indicus*[6] (Beng. Chagal nadi) etc. are used as cure of jaundice.

o. Malaria

An infusion of the flowers of *Caesalpinia pulcherima*[14] (Beng. Krishnachura) in malarial fever, ground leaves of *Calycopteris floribunda* Linn.[4] (Beng. Goachelata),leaves of *Helianthus annus*[4] (Beng. Surjamukhi), the decoction fo *Lantana camara* Linn[4] (Beng. Chotra) and bark of *cincona* etc. are considered useful in the treatment of malaria.

p. Rheumatism

The plant of *Vitis pallida*[21] W & A prodr. The Leaves of *Acanthus ilicifolius*[4] (Beng. Harzora, Kotki, harkuch), *Allium Cepa*[4] Linn. (Beng. Piyaj), *Alpinia nigra* (Beng. Tara, Jangliada), *Azadrachta indica* (Beng. Nim), *Coriandrum sativum*[4] (Beng. Dhane, Dhanya), *Dipterocarpus alatus*[4] (Beng. Garjan, Shilgarjan, Dhuligarjan, Mashkalya garjan) etc. are used as against rheumatism.

q. Skin diseases

The sap of the young branches of *Vitis vinifera*[21] Linn. (Beng. Angurphal). The flowers of *Helianthus annus*[6] (Beng. Surjamukhi), the leaves and barks of *Lawsonia inermis*[6] (Beng. Mehidi), *Hydrocotyl asiatica*[6] (Beng. Thankuni), the barks of *Albizzia amara* (Beng. Amlaki), the plants of *Cynodon dactylon*[21] (Beng. Durba), the barks, leaves and juice of ripe fruits of *Cassia fistula* Linn.[6] (Beng. Badar Lathi) etc. are used as medicines for the treatment of skin diseases.

r. Tonics

The flowers of *Helianthus annus*[6] (Beng. Surjamukhi), *Rosa centifolia*[6] (Beng. Golap), the leaves and flowers of *Acacia arabica* (Beng. Babla), *Psidium guyava*[6] (Beng.Beng. Peyara, Piyara), the seeds of *Carina papaya*[6] (Beng. Papaya), *Trigonella foenumgraecum* (Beng.Methi)[16], the fruits of *Terminalia chebula*[6]

(Beng. Hortoki), the barks of *Termenalia arjuna*[6] (Beng. Arjun, Arjuna), the plants of *Vernonica cinerea*[6] (Beng. Kalajira) etc.are used as tonic

s. Typhoid

Celerodendrum inerme[4] (Beng. Bhant, Koklata,Banjui, Batrag, Bakri), bark and roots of *Croton oblonjifolius*[4] (Beng.Chuka, Patri, Baragachi), *Desmodium gangeticum*[4] (Beng. Salpani, Chaloni), Grewia microcos[4] (Beng.Asar,Patka), hedyotis corimbosa[4] (Beng. Khetpara), *Uraria lagopoides*[4] etc. are used for the treatment of typhoid (remittent).

t. Ulcers

The stem of *Vities quadrangular*[21] *is* wall (Beng. Harzora lata) is used for chronic ulcers.The leaves of *Psidium guyava*[6] (Beng. Payara), *Lawsonia inermis*[6] (Beng.Mehidi), the barks of *Acacia farnesiana*[6] (Beng. Guya babla), *Acacia catechu* (Beng.Khoyer), *Eugenia jambolana*[6] (Beng. Jam), *Punica grantum*[6] (Beng. Dalimagach) *Terminalia arjuna*[6] (Beng.Arjun), the plants and roots of *Ipomoea turpethum*[3] (Beng. Dudkalmi), the leaves and fruits of *Areca catechu*[6] (Beng. Shupari) etc. are used as medicine for the treatment of ulcers.

1.2 Aim of the present work :

The present project was designed to isolate pure compounds and determine the individual structures of the isolated compounds.

From a through literature review it appears that there is a lot of medicinal and economical application of this plant. So on the basis of its uses and properties, we have taken further attention to carryout a chemical investigation of the plant *Vitis quadrangularis* wall. which is also named as *Cissus quadrangularis* linn. of vitacae family. The present work aims at the isolation, purification and structural elucidation of individual compounds from the extracts of this plants.

As rural people use its leaves, stems and roots in different forms for alleviation of bone fracture, wounds, paste of stem is given in scurvy and asthma[31,32,33]. So it is highly expected that some biologically active compounds might be found in the individual form from solvent extracts of the stem of *Vitis quadrangularis* wall.

The project consists of the following steps :

(i) Extraction of the stems of plant *Vitis quadrangalaris* wall. with organic solvents of increasing polarity e.g. Petroleum Ether (b.p. 60-80°), Chloroform ($CHCl_3$), and Methanol (MeOH)

(ii) Use of TLC for monitoring

(iii) Fractionation of crude extracts by ColumnChromatography (C.C)

(iv) Isolation and purification of the compound.

(v) Determination of the structures of the isolated compounds with the help chemical and physical method e.g., IR, NMR, GC-MS etc.

1.3 DISCUSSION ABOUT VITIS AND CISSUS :

In 1967 the taxonomist Jain named the plant Harzora lata or Harvangha lata as *"Cissus quadrangularis"* Linn and later on in 1982 another two taxonomist Nazimuddin and Qasir[35] named as *Vitis quadrangularis* wall of vitaceae family of genus vitis.

1.4 DESCRIPTION OF PLANTS OF THE VITIS GENUS :

Vitis is an important genus of vitaceae family [21]. Small trees or climbing shrubs, the latter usually tendril bearing, steams and branches nodose. Leaves alternate, simple lobed digitate or pedate, sometimes pinnate or bipinnate ; petiole usually thickened at the articulate base ; stipules 2. Flowers regular, hermaphrodite or unisexual, in panicled umbelled or spicate cymes usually opposite the leaves, peduncles often transformed into tendrils or tendril-bearing. Calyx small, entire or 4-5 toothed or lobed. Petals 4-5, valvate, free or connate, caducous. Disk free or connate with the petals stamens or ovary, annular or expanded. Stamens 4-5, opposite the petals, inserted at the base of the disk or between its lobes ; filaments subulate ; anthers free or connate, 2-celled, introrse.

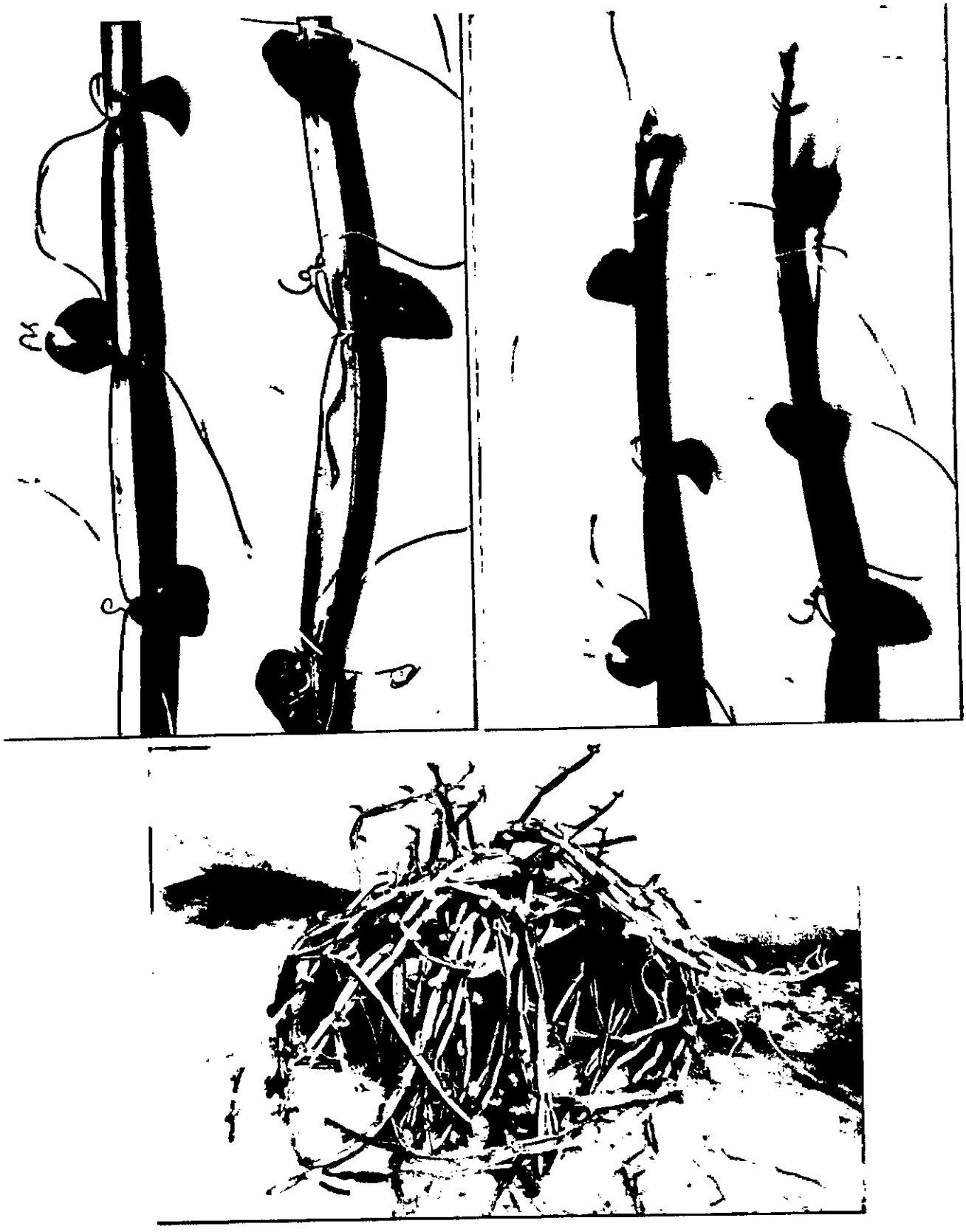


Fig. (1) ***Vitis quadrangularis*** **Wall. (Harzora Lata)**

Ovary usually sunk in the disk, 2-6-celled ; ovules 1-2 in each cell, ascending, anatropous ; style short ; stigma small, capitate or slightly lobed.

Fruit an indehiscent 1-6-seeded berry, often watery.

Seeds erect, often rugulose ; albumen cortilaginous sometimes ruminate ;
embryo basa ; cotyledons ovate or cordate ; radicle short, inferior.

There are 11 genera and 450 species in this genus [23](iv) They are chiefly cooling and bechic.

1.5 GENERAL DESCRIPTION OF VITIS QUADRANGULARIS PLANT :

Botanical Name : ***Vitis quadrangularis (Wall) also ndamed as Cissus quadranguaris (Linn)***

Family : Vitaceae

Genus : Vitis

Local Name : Harzora lata, Harvangha lata

English Name : Granadilla

DESCRIPTION :

Harzora Lata [27](Vitis quadrangularis, wall) A climbing type wild vine with quadrangular stem and sympodial branching, grows in the Sundarbans and occasionally planted in gardens in other areas. This plant also grows in other parts of Indian sub-continent. [25]It is a well known medicinal plant of this sub-continent.

Stems leafless when old, very long, fleshy, glabrous, much contracted at the nodes, quadrangular, the angles of the young branches winged ; tendrils long, slender, simple. Leaves 2.5- 5.0 cm. long, broadly ovate or reniform, sometimes 3-7-lobed, denticulate, glabrous, cordate, rounded, truncate or cuneate at the base ; petioles 6-12 mm. long ; stipules small, broadly ovate, obtuse[21,22,23].

Flowers in shortly peduncled cymes with spreading umbellate branches. Calyx cup-shaped, truncate or very obscurely lobed. Petals 4, ovate-oblong, acute 3 mm. long, hooded at the apex. Disk erect, 4-lobed. Style short, stout. Berry obovoid or globose, scareely 6 mm. long, apiculate, red when ripe, 1- (very rarely 2-) seeded.

1.6 MEDICINAL USES OF DIFFERNET SPECIES OF GENUS VITIS :

Vitis adnata Wall :

The dried tuber is used by the country people as an alterative, in the form of a decoction ; they consider that it purifies the blood, act as a diuretic and renders the secretions healthy.
The root, powdered and heated, is applied to cuts and fractures [26]by the santals.

Vitis latifolia Roxb :

It is a sub-Himalayan plant. The roots of this plants are used to wounds.

Vitis vinifera Linn. :

It is a large deciduous climber plant. It has five kinds of fruit , the ripe fruit is rid ; cooling, laxative and purgative, fattening, diuretic, aphrodisiac, appetiser, very good for the eyes, and the throat ; cures thirst, fever, asthma, "vata" and "vatarakta", jaundice, strangury, burning bad effects of drinking, blood diseases ; vomiting ; difficult to digest, causes of gases in the stomach, causes of "kapha".
The sour fruit causes biliousness. The black fruit is aphrodisiac ; cures "kapha" and biliousness (Ayurveda).

The fruit is sour, sweet ; digestive, stomachic, expectroant ; purifies and enriches the blood ; good for lungs, liver and kidney ; fattens the body ; very useful in old fevers ; it is recommended to weak people. The dried fruits are demulcent, laxative, sweet, cooling, agreeable and usefal in thirst, heat of body, cough, hoarseness and consumption.

The leaves are useful in piles. There juice cures headache, syphilis, piles, inflammation of the spleen ; diuretic ; allays vomiting, stops bleeding from the mouth ; applied in scabies ; produces alopecia. The leaves, on account of their astringency are sometimes used in diarrhoea.

The ashes of the stem are good for pains in the joints, stones in the bladder, swelling of the testicle and piles.

The flower is expectorant, emmenagogue ; enriches the blood ; tonic to the liver ; it is very good in chronic bronchitis ; produces constipation.

The sap of the young brances is a popular remedy for skin diseases, and is still a popular remedy in Europe for ophthalmia.

The seeds are cooling, aphrodisiac, astringent to the bowels ; their ash is applied to diminish inflammation (Yinani).

The juice of the unripe grapes is used as an astringent in affection of the throat. Black raisins in combination with other drugs are prescribed for the treatment of snake-bite (Sushruta, Vagbhata) and scorpionsting (Sushruta). Raisins are not an antidote to snaka venom (Mhaskar and Caius) or to scorpion venom (Caius and Mhaskar).

In modern native practice, the raisins are considered cool and aperient, and given in coughs, catarrh and jaundice.

Vitis indica W. Arn. :

The juice of the root, with the kernel of the cocoanut, is employed as a depurative and aperient. In the konkan, the country flk use it as an alterative in the form of a decoction, and they consider it to purify the blood and act as a diuretic and render the secretions healthy.

In cambodia, the roots are considered pectoral and diuretic ; they are used in bronchitis and gonorrhoea.

Vitis setosa Wall. :

The leaves are a useful local stimulant and are much used as a poultice to promote suppuration. This is also applied externally to assist in the extraction of the guinea-worm.

Vitis carnosa Wall. :

The root has a sharp sour taste ; cures "Vata" and "Kapha" tumours, pains, and spleen complaints (Ayurveda). The juice of root is sour with a sharp taste ; purifies the blood and lessens biliousness; it is good for liver and heart troubles and for inflammation of the spleen; produces cough (Yunani).

The root is used as an astringent medicine. Stewart remarks that the root, ground with black pepper, is applied to boils.

The names given to it in many parts of India denote one of its most general uses, viz., the treatment of yoke sores on the necks of bullocks. For that purpose a poultice of the leaves is employed (Elliot).

According to Irvine the seeds and also leaves are employed as an embrocation.

Vitis araneosa Laws. :

The tuberous starchy, astringent roots, sliced and dried, are sold by the konkan herbalists, under the mane of chamar-musli.

Vitis pedata Vahl ex Wall. :

Throughout India, Ceylon and Malaya, sometimes used as a substitute for V. Setosa. This plant is used as a domestic medicine, because of its astringency.

Vitis tomentosa Heyne in Roth. :

With the Santals the root is deemed useful to allay swellings.

Vitis repens Wight & Arn. :

This plant is applied to shoughing and foetid ulcerations, also to boils and small abscence as maturant.

Vitis pallida W & A prodr. :

It is a climbing shrub. This plant is used for remedy of rheumatism.

1.7. Medicinal uses of genus *Vitis quadrangularis* Wall. (Cissus quadrangularis Linn.) :

Vitis quadrangalaris Wall. :

The stem is hot, dry, sweetish, bitter ; laxative, anthelmintic, digestible, aphrodisiac, stomachic, tonic, analgesic ; it removes "Vata" and "Kapa", piles, blindness, epileptic fits, tumours, loss of appetite and constipation ; cures eye diseases, chronic ulcers ; it is good for the spleen ; benefical in fractures [26]of the bones and in ascites ; causes biliousness (Ayurveda).

The stem is bitter ; it is given internally and applied topically for broken bones ; it is used in complaints of the back and spine ; removes pus (Yunani). The stem beaten into a paste is given in asthma boiled in lime water it forms a preseve useful as a stomachic. The Rongas of East Africa apply the pounded stem to wounds[21].

The Juice of the stem is useful in scurvy and in irregular menstruation . It is given in otorrhoea and in epistaxis.

The leaves and young shoots are powerful alteratives ; dried and powdered leves are administered in certain bowel affections connected with indigestion.

1.8 ECONOMICAL IMPORTANCE OF "VITIS QUADRANGULARIS" WALL & ITS DIFFERENT SPECIES :

Medicinal plants are rich sources of bioactive compounds and thus serves as important raw materials for drug manufacturing. There are many countries in the world which earn a lot of foreign currency by exporting medicinal plants as well as crude plant drugs. India and Thiland are two excellent examples of such countries in the subcontinent. There are still other countries such as China, India and Pakistan which utilize their own medicinal plants for local manufacture of both Eastern and Western medicines and pharmaceutical products. In contradiction, Bangladesh in spite of having a large flora of medicinal plants every year imports a huge quantity of pharmaceutical raw materials including medicinal and semi-processed plant products to feed its various drug manufacturing industries. According to the herbal medicinal sources the herb *Vitis quadrangularis* [28] Wall. have multiple medicinl applications. The herb contains [31] an oxo-steroid, similar in action to durabalin, 3-Ketosteroid, other steroids and ß-sitosterol, carotene,ascorbic acid and calcium oxalate. All these compounds are used as medicine.

Besides this, the vitaceae is a family of mainly climbers and some shrubs cele-brated on account of one species, Vitis vinifera, the grapevine, ageold provider of wine and fruit. Severl genera are grown as ornamental creepers [28] e.g. cissus, parthenocissus and Vitis itself.

Economically the vitaceeae [23]is important because of the grapevine (Vitis vinifera) which originates from the orient and northwest India. More than 25 million tonnes of wine are made annually from the fruit of this species, and viticultare is now a scientific study. When dried, the fruits are termed raisins, or sultanas if the grape is of the seed less variety. Currants are the dried fruits of the corinthian variety. Grapes of the Muscatel variety are used to make the wine that name, as well as raisns. The fruits of some other species of vitis e.g V. aestivalis and V. labrusca are also used for wine-miking. These are North American species which are resistant to the insect phylloxera. On account of the devastation caused by this pest, most European vines are now grafted on to American root stocks. The stem of some species, e.g. V. papillosa (Java) and V. sicyoides (Mexico) are used localy as cordage.

Some other members of the family are prized as ornamentals, notably the 10 species of parthenocissus (Virginia creeper). All are climbers, Parthenocissus quinquefolia or "true" virginia creeper, possessing leaves whith three or five coarsely serrated leaflets which turncrimson in autumn. This and other species of parthenocissus, e.g P. inserta and P. himalayana, are suitable for covering walls, fences and pergolas, as are some species of vitis such as V. amurensis and V. davidii and of Cissus.

1.9. CHEMICAL STUDIES OF THE PLANTS OF VITIS GENUS :

In 1981, Brieskorn, C.H and his co-workers isolated[30] a new product from a very well-known species of vitaceae family Vitis vinifera from methanollic extraction. The structures of the compund was determined by using the PMR, CMR spectral analyses. The compounds name 16-HyroNy-3-oxo-1, 12-oleanadien-28 oic acid is shown below.

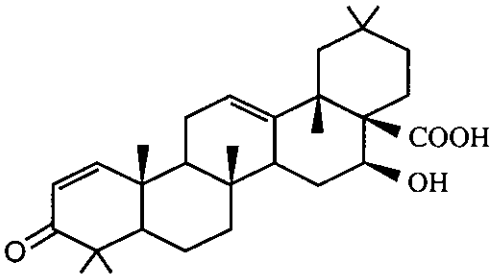


Fig. (2) 16-Hydroxy-3-oxo-1, 12-oleanadien-28 oic acid.

In 1983, Karl, Christian and his co-workers worked [29]on the plant *Vitis vinifera* of vitaceae familly. There were many isolates from methanolic extracts of dried leaves[29] of *vitis. vinifera* and 3-ellagitanins were isolated ; brevilagin 1,-3-digalloyl-4, 6-dehydrohexahydroxydiphenonyl glucose (vitilagin) and 3,4-digalloyl-1, 6-dehydrohexahydroxydiphenoyl glucose (isovitilagin).

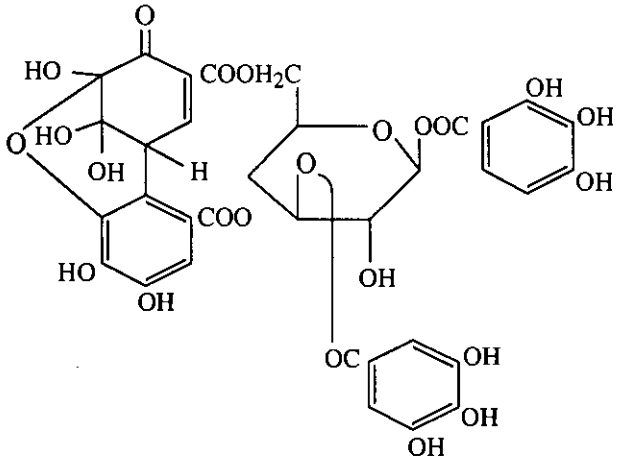


Fig. (3) 1,-3-digalloyl-4, 6-dehydrohexahydroxydiphenonyl glucose (vitilagin)

In 1993, Oshima, Y and his co-workers [28] isolated various compounds from methanolic extract of one of the common vitaceaeous plants *Vitis coigenetiae*, showed marked prevention against injuries of primary cultured rat liver cells induced by carbontetrachloride and D-galactosamine. Activity guided fraction of the extract resulted in the isolation of not only an antihepatotoxic stilbene deriv., Є-viniferin, but also novel oligostilbenes, vitisin-A (I)and its stereoisomer cis-vitisin A (II) as a mixt. the structures [24-27] of the oligostilbenes were determined on the basis of spectroscopic evidence esp. by 2D NMR method such as HMBC spectra of degradative products. The mixt. of I and II was found to cause merked liver lesions in mice.[28]

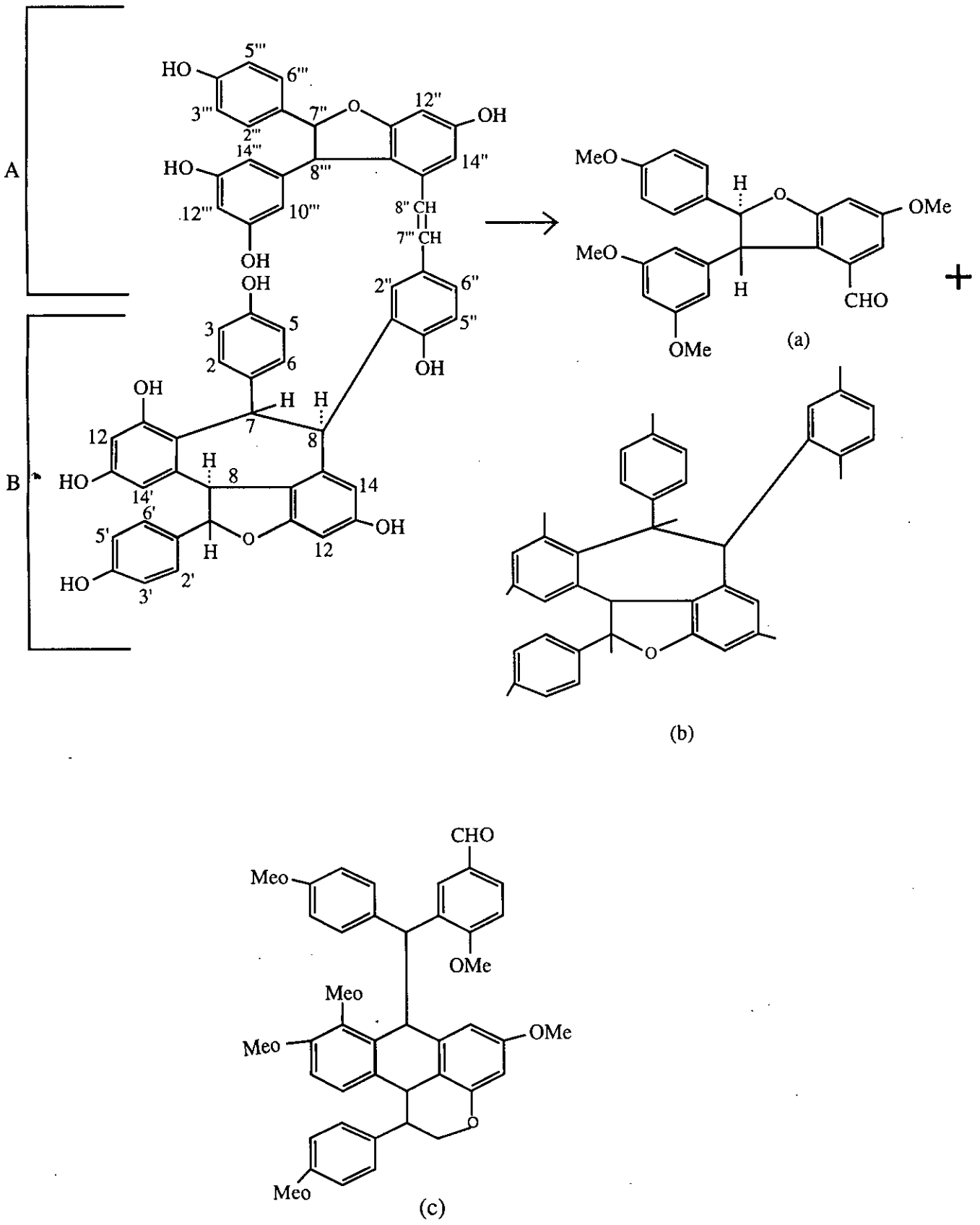


Fig. (4) Vitisin A, Cis-vitisin A.

In 1995, Oshima, Y and his co-worker also worked on the same plant of vitacea family. *Vitis coignetiae*. They isolated another compound, vitisin B, molecular formula $C_{56}H_{42}O_{12}$ and molecular weight 906.941. It is also named as cis-vitisin B. The structure was determined[34] by using UV, IR, PMR, CMR and MS, spectra.

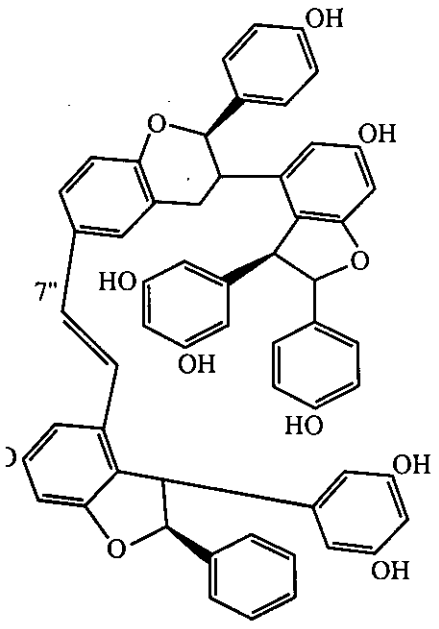


Fig. (5) Vitisin B.

1.10 CHEMICAL STUDIES ON *"VITIS QUADRANGULARIS".*

From a thorough literature review it appears that a few chemical investigation has been done on leaves, roots and stems of *Vitis quadrangularis*. Prior to this study. The available chemical reports of this plant *Vitis quadrangularis*. are given below.

In 1984, Bhutani, K. K. and his co-workers isoletated 7-Onocerene-3, 21-diol from methanolic extact of *Cissus quadrangularis* L. which was named as *Vitis quadrangularis* Wall. from The melting point was recorded as 233-234°C and its structure were determined by chemical and spectral method, which is shown below.

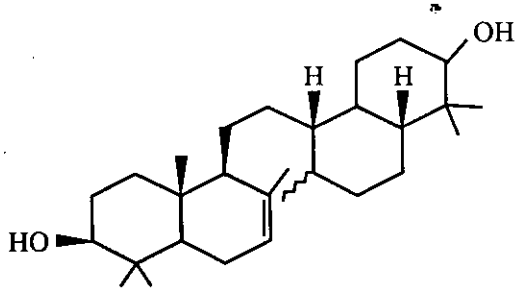
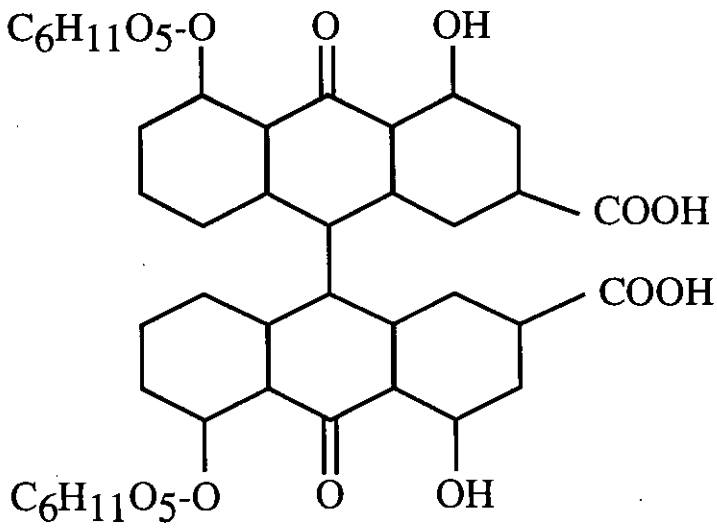
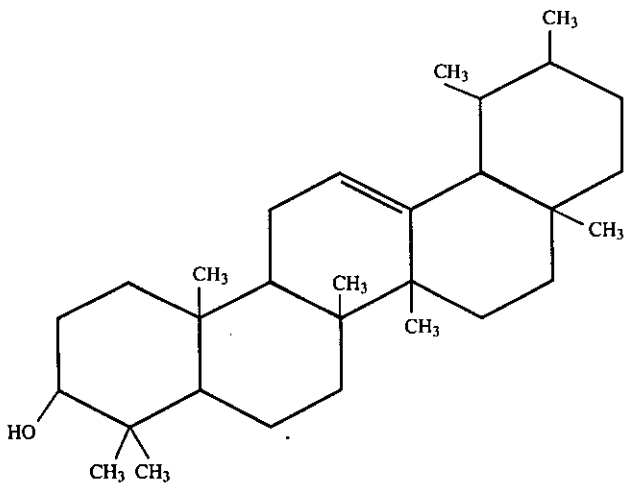


Fig. (6) 7-Onocerene-3, 21-diol

It has been reported [41] that from the plant the triterpenoids I (R# R^1 = ∞-OH) were isolated from ethanolic extracts from *Cissus quadrangularis*. and their structures were determined by chemical and spectral methods. sitosterol, ∂-amyrin, and amyrone were also obtained.



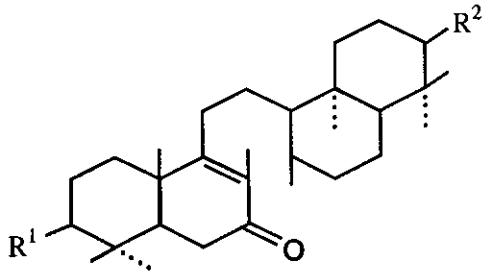
a. Amyron



b. ∞-Amyrin

Fig. (7) : a. Amyron, b. ∞-Amyrin

In 1990 M. M. Gupta and R. K. Verma worked [36,37,38,39,40] on *Cissus quadrangularis* Linn and isolated two new unsymmetric tetracyclic triterpenoids, Onocer-7-ene-3∝, 21ß-diol and Onocer-7-ene-3ß, 21∝diol together with ∝amyrin and ∝anyrone from the plant [24]. For this investigation the dried and powdered aerial parts of *Cissus quadrangularis* was extracted with EtOH (5X61) by cold percolation and the extract was concentrated in vacuum, diluted with water and extracted with n-hexane (5X500 ml), $CHCl_3$ (5X500 ml), EtOAC (5X500 ml), and n-BuOH (5X250 ml), respectively. The residue was chromatographed over silicagel. Elution was crried out in varying percentage of hexane in C_6H_6 and C_6H_6 in $CHCl_3$. Fractions were collected and monitored by TLC. Removal of solvent from fractions of (3:1, hexane : C_6H_6) afforded a residue, of melting point 235-237° (Me_2CO), and was identified as 7-Oxo, onocer 8-ene-3ß, 21∝diol (Ia).

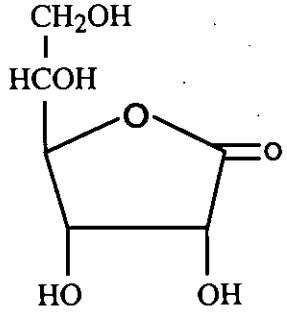


Ia R^1 = ß.OH. R^2 = æ-OH
Ib R^1 = ß.OAc. R^2 = æ-OH
Ic $R^1 = R^2$ = o

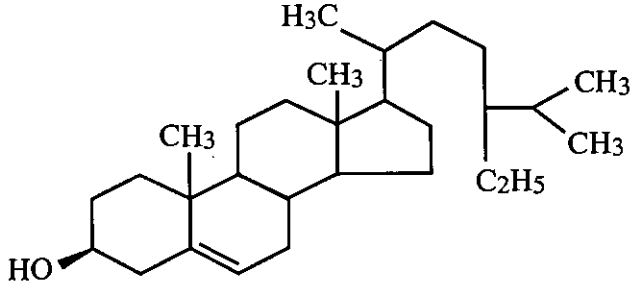
1990

Fig. (8) 7-Oxo, onocer 8-ene-3 ß, 21∝diol (Ia).

Besides this 1998, Abdul Ghani compiled a book entitled Medicinal plants of Bangladesh. The herb *Vitis quadrangularis* wall, contains an oxo-steroid, similar in action to durabalin, 3-ketosteroid, other steroids and ß-sitosterol, carotens, ascorbic acid and calcium oxalate [31]. The structure of these compounds are cited below :



a. Ascorbic acid.



b. ß-sitosterol.

Fig. (9) a. Ascorbic acid. b. ß-sitosterol.

1.11 BIOSYNTHESIS OF TERPENOIDS

INTRODUCTION :

The terpenes are a unique, highly diverse group of compounds. They are structurally diverse, yet a thread of commonality concerning their biosynthetic origin allows many apparently unrelated compounds to be viewed unifying prspective. Some illustrative examples will be presented shortly.

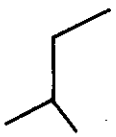
The terpenes have held a special interest to organic chemists for nearly 100 yers,Indeed, the names of many great organic chemists are associated with this area of research ; they include Perkin, Baeyer, Wieland, Meerwein, Karrer, Butenundt, Ruzicka, Doisy, Sir Robet Robinson, Reichstein, Diels, Alder, Bloch, Lynen, Sir Derek Barton, Sir John Cornforth, Prelog and of course Woodward. The name of woodward, like that of sir Robert Robinson, will come up time and again in this book. The composition of the main terpene groups increases from a five - carbon unit by five - carbon units :

C_5	Hemiterpene	C_{20}	Diterpene
C_{10}	Monoterpene	C_{25}	Sesterterpene
C_{15}	Sesquiterpene	C_{30}	Triterpenes.

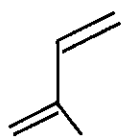
The five-carbn unit has the 2-methyl butane structure and is colloquially known as the isoprene unit (a). It is not uncommon to find the terpenes referred to as "isoprenoids.

A characteristic of many terpenes is that their skeleta can be dissected in terms of five carbon units having the isoprene skeleton. When that is possible the compound is said to "follow" the isoprene rule. But how did these compounds come to be regarded and grouped in this way ?

In all fairness it cannot be said to have started with Faraday, who deduced that rubber was a polyunsaturated polymer of pentadine having the molecular formula $(C_5H_8)_n$. Carotene was subsequently shown to be a $(C_5)_n$ compound, and Whallach determined that some volatile terpene hydrocarbons had the molecular formula $C_{10}H_{16}$ whereas others had a formula $C_{15}H_{24}$. Several other workers were also busy investigating the nature of rubber and found that pyrolysis afforded isoprene (b), which could be polymerized to rubber.



a. isoprene unit



b. isoprene

However it was Wallach who in 1887 wrote ; "Such a structure for isoprene ..... allows a polymerization to terpenes, sesquiterpenes etc., to appear reasonable ....." Wallach then went on to delineate some eamples of his ideas. Although the structures were not correct, the idea was there, As with many good ideas it remained dormant for a long period even as more and more structures were being elucidated. Ruzicka over a period of many years is responsible for the development of the isoprene rule, which could assist in the elucidation of new structures and correct previously suggested structures.

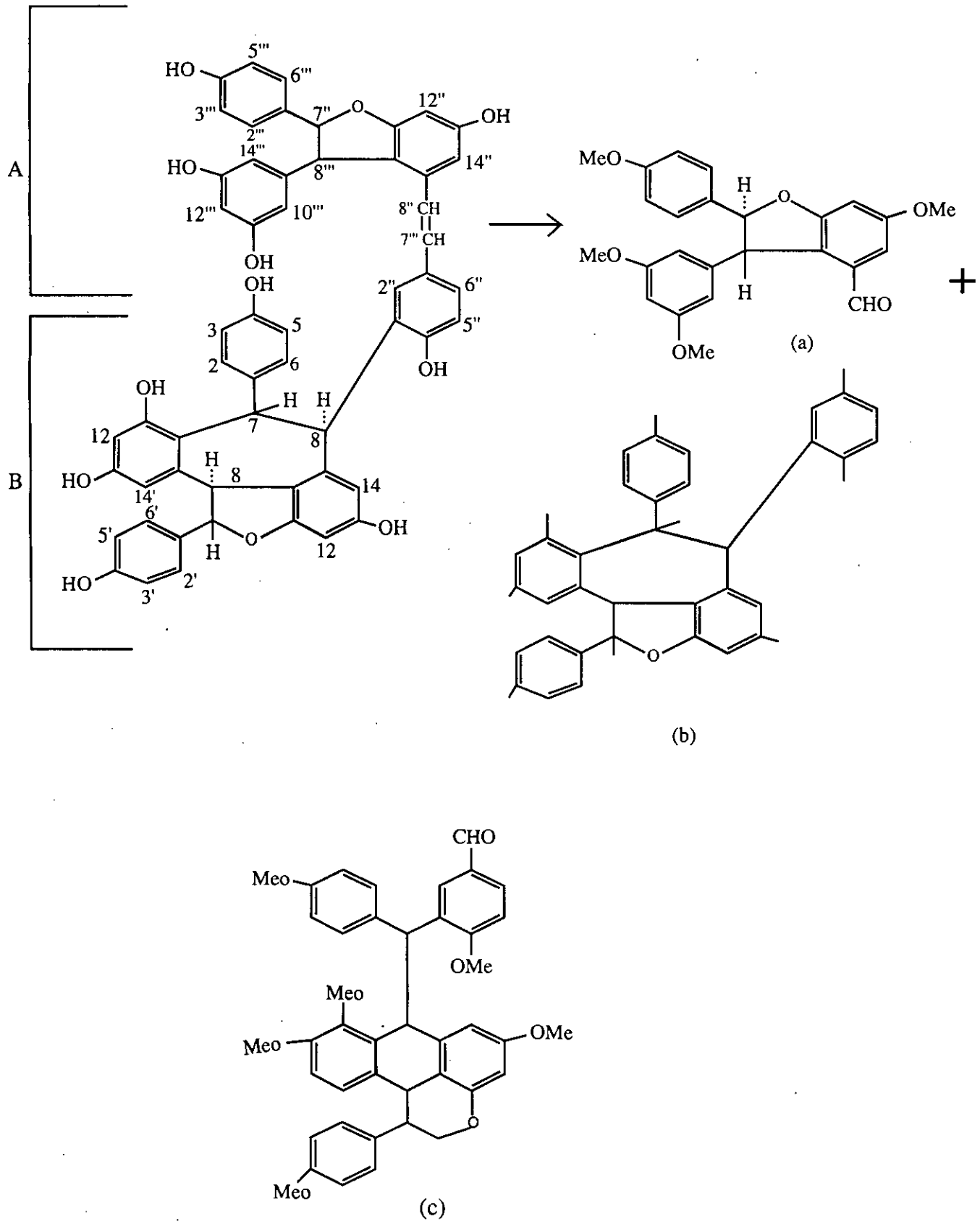


Fig. (4) Vitisin A, Cis-vitisin A.

Farnesyl pyrophosphate (g) may also add another IPP unit to give the precursor of the diterpenes, geranylgeraniol phyrophosphate (h). Alternatively, two farnesyl units may join together to afford squalene (i), the precursor of the triterpenes and ultimately the steroids. Let us focus in on the rearrangement of squalene to the triterpeoids.

REARRANGEMENT OF SQUALENE TO THE TRITERPENES :

Squalene, as indicated previously, is the C-30 compound which is a precursor of all triterpenods and steroids. The steps from squalene to the steroids in mammals and plants have been and continue to be the subject of numerous studies. A detailed discussion of these results is beyond the scope of this book and the interested reader is referred to the most recent treatment of this subject by Nes and Mckean for additional in formation. Here we will discuss only the quite basic details of this complex scheme.

In 1934, Robinson indicated how a particular conformation of squalene could lead to lanosterol. When the structure of lanosterol was finally proven in 1952, the unique talents of woodward and Bloch combined to suggest a cyclization of squalene which differed sinificantly from Robinson's in that the gem-dimethyl group at C-4 is derived directly from the terminal carbons of squalene and not by subsequent alkylation.

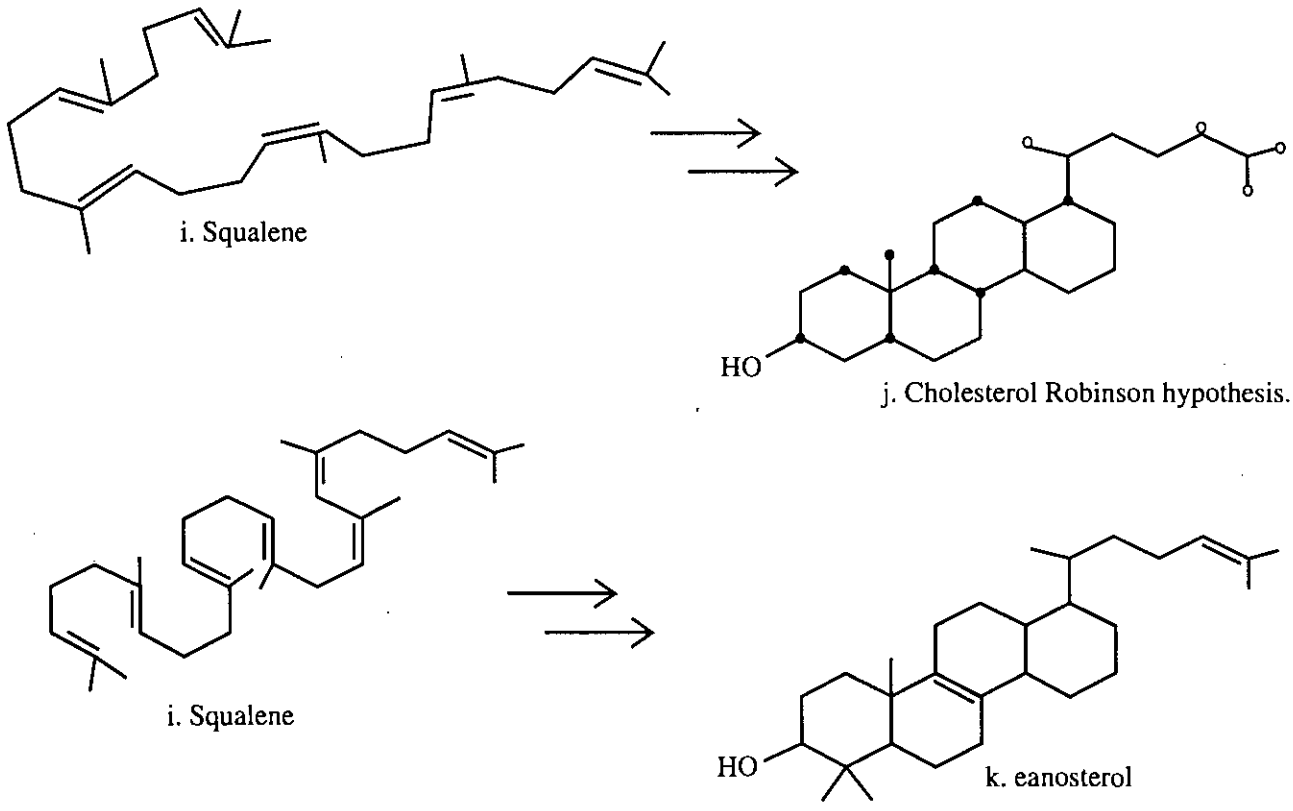
The schemes of Robinson and of Woodward and Bloch are shown in scheme-2 , indicating the positions expected to be labeled by the methyl group of acetate. As a result, the two labeling patterns the same carbon atom may come from a different source. The carbon atoms of interest in this respect, and thus those which could serve to distinguish between the two schemes, are C-7, C-8,C-18 and C-13 of cholesterol. Experiments with labeled acetate established that C-13 was derived from the methyl group of acetate, indicating that the Wood-ward-Bloch hypothesis, in which lanosterol (k) is an intermediate, was correct. All subsequent experiments with labeled acetate, mevalonate, and farnesol have substantiated this pattern of folding for the formation of the titerpene nucleus.

A very important difference bet-ween diterpenes and triterpenes should be mentioned at this point. Whereas the latter almost always have an oxygen functionality al C-3, such a group is quite rate in the diterpenes.

Bloch determined tht the oxygen atom of this hydrony group was derived from molecular oxygen, and for some years it was felt that + OH, produced by reduction of molecular oxygen by a reduced pyridine nucleotide (e.g,. NAPPH), was responsible for the initiation of cyclization. The groups of Corey clayton, and van Tamelen however established that the processes of oxidation and cyclization are separate steps involving squalene-2, 3-oxide (l) as an intermediate. Some of this evidence includes : (a) when 2, 3-imino squalene is used as an inhibitors of the cyclization, k accumulates ; (b) squalne-2, 3-oxide (l) was converted by rat liver homogenates to lanosterol (k) and other products ; and (c) the oxygen atom of (l) is the same oxygen as appears at C-3 in lanosterol (k). Additionally it has been found that the S-isomer of k is the sole precursor of lanosterol (k) in yeast and pig liver and of ß-amyrin, lupeol, and cycloartenol (m) in Pisum sativum (Pea) seedlings.

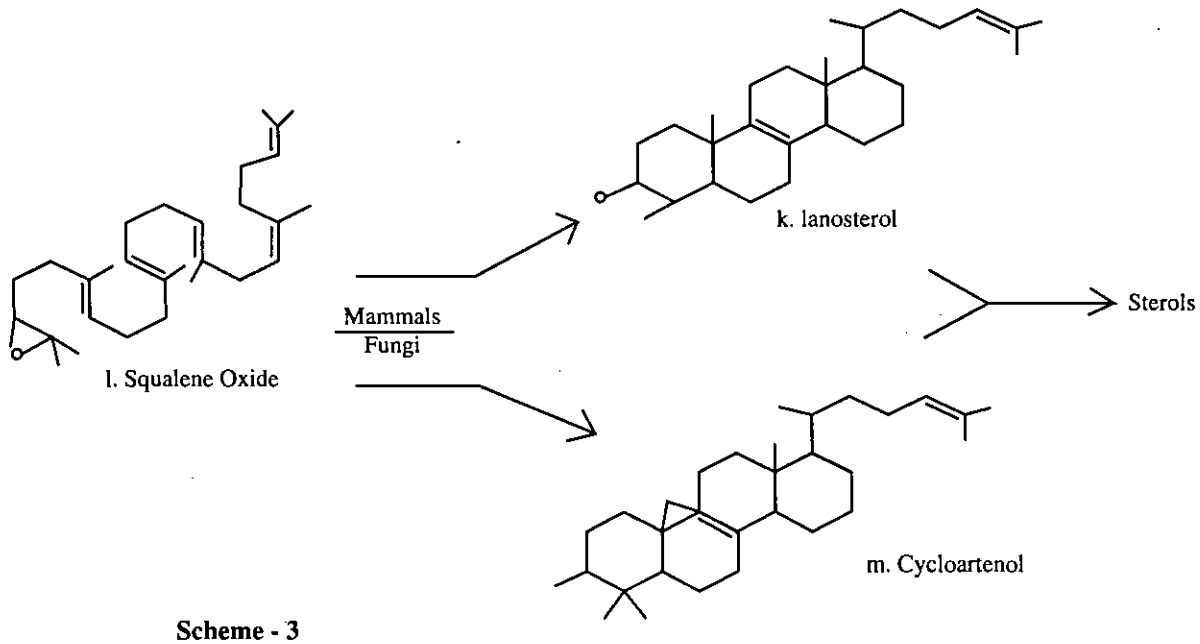
The cyclizations of squalene oxide (l) and the subsequent rearrangements are a very compex subject and the readerare referred to Nes and Mckean for an extensive discussion.

One clear point should be made at this early stage, namely, that in mammalian systems and in most fungi.



Scheme (2) : Woodwoard-Bloch hypothesis

Lanosterol (k) is the intermediate to the steroids. photosynthetic plants however, it is cyeloartenol (m) which is the precursor of setroids, schem (3).

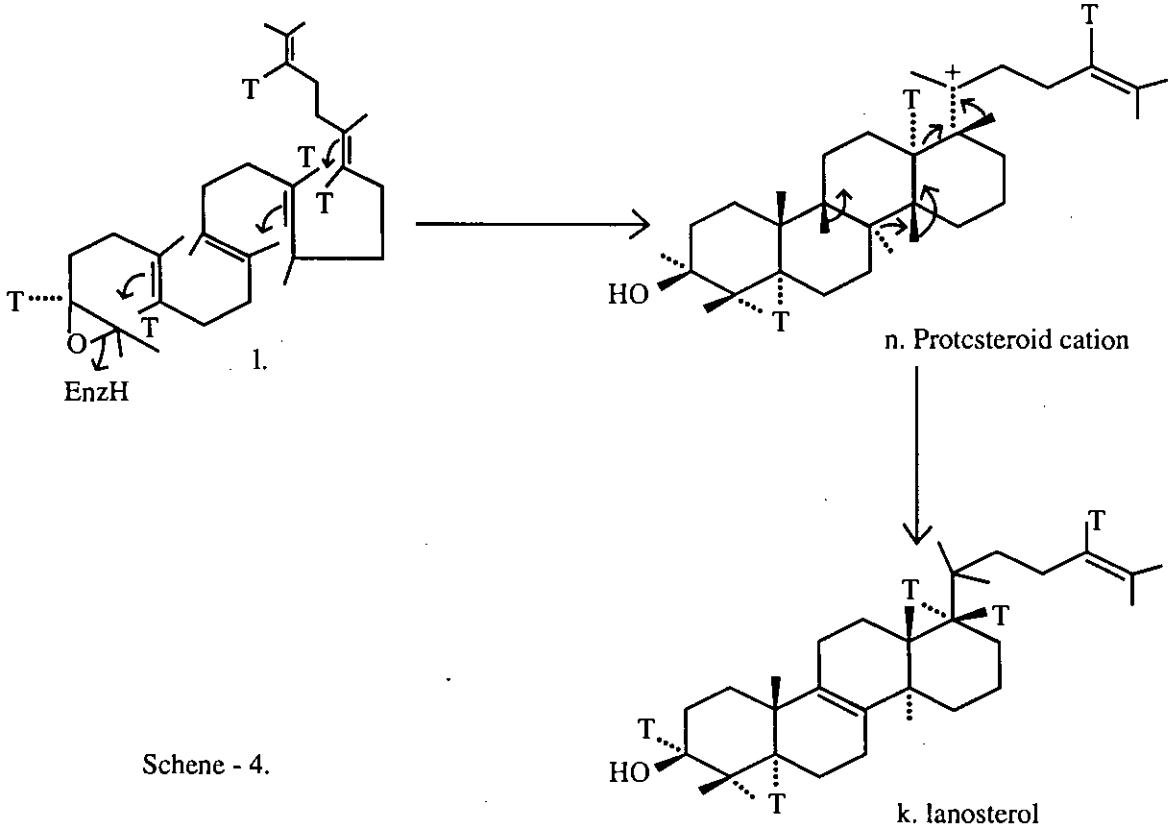


Scheme - 3

At this point attempt should be made to define the six isoprene units present in the nuclei of cycloartenol (m) and anosterol (k). No matter what end you start from, neither compound obeys the biogenti isoprene rule. The methyl group at C-13 should be at C-8. Evidently at a very early stage a rearrangement has taken place for, as shown in scheme 3, the initial product of cyclization of sqnalene oxide (l) should be the cation (n), the SO-called protosteroid cation. Also note that compared to the typical stereochemistry of cycloartenol (l), several of the ring functions have their stereochemistry inverted.

It is at the point of the protosteroid cation (k) where rearrangement takes place on one hand to lanosterol (j) and on the other hand to cycloartenol (m).

The mechanism of the rearrangement to lanosterol (k) is shown in scheme (4). Initiation of what is apparently a concerted process is by enzyme removal of a proton at C09, This begins a series of suprafacial shifts in which a new double bond is introduced at $4^{8,9}$, the C-8 methyl migrates to C-14, te C-14 methyl to C-3, and hydrogens at C-13 and C-17 migrate to C-17 and C-20 respectively. when [$4R^3$-H] mevalonic acid is used as a precursor of lanosterol (j), only five of the possible six times are incorporated as expected in this scheme.



Schene - 4.

The rearrangement to cycloartenol (k) is thought to produce not by proton loss from C-9 but rather by hydride shift from C-9 to C-8 with stereo specific complexation of an enzyme at C-9 to give an intermediate such as 11B. Loss of proton from the methyl group at C-10 with displacement of the enzyme gives cycloartenol (l).

CHAPTER - 2

CHAPTER -2

EXPERIMENTAL

2.1 GENERAL METHODS :

During the present investigation solvents were purified by distillation at the boiling point of the respective solvents. Evaporation of the solvents was carried out by a rotary vacuum evaporator under reduced pressure at a temperature below 45°C. The purity of the isolated compound was tested by analytical thin layer chromatography (TLC) and the spots were detected by a spray reagent followed by heating the plate in an oven for ten minutes. The crude was subjected to column chromatography over (230 mesh) silica gel to obtain pure compound.

2.1.1. MELTING POINT :

Melting points were recorded by Fisher John's electrothermal melting point apparatus by using thin disc method. The heating was applied carefully to ensure a steady rise of temperature.

2.1.2. THIN LAYER CHROMATOGRAPHY (TLC) :

Thin layer Chromatographic method was used for the analysis of the different mixtures of compounds and also to determine the purity of the isolated compounds.

Thin layer chromatography was carried out on glass plates which were coated with a layer of silica gel (60 GF 254'EMERCK) and were activated by drying in an oven before use. The glass plates were thoroughly washed with water and acetone and dried. With the help of a spreader, the glass plates were coated with a layer (thickness 0.02 mm) of silica gel by spreading an emulsion of silica gel (about 8.0 gm silica gel in 16.0 ml water). The plates were then allowed to stand for two house for drying at room temperature and warmed in an oven at 110°C before use.

More' frequently precoated TLC plates with silica gel 60, Kieselguhr F 254 (thickness 0.2 mm. E. MERK) on aluminiumum foils were used.

2.1.3. COLUMN CHROMATOGRAPHY (CC) :

The technique of column chromatography was used to separate the individual components of a mixture having different R_f values. The chromatographic column was prepared by slurry method using silica gel (kiesel gel 60, 230 mesh, ASTM, E. MERK) as the stationary phase and freshly distilled solvents were used for elution, of the crude on TLC plates in various solvents. Eluents were-collected and examined by TLC to monitor the separation.

2.1.4. IR (INFRARED SPECTRA) :

Infrared spectra were recorded on a SHIMADZU FTIR DR- 8101 Spectrophotometer in KBr at the Department of Chemistry, Dhaka University, Dhaka.

2.1.5 ^{1}H-NMR SPECTRA :

^{1}H NMR Spectra were recorded in $CDCl_3$ using 400 MHz spectrophotometer at BCSIR, Science laboratory, Dhaka.

2.1.6. ^{13}C-NMR SPECTRA :

^{13}C-NMR spectra were recorded in $CDCl_3$ using 400 MHz spectrophotometer at BCSIR Science laboratory, Dhaka.

2.1.7. GC-MASS SPECTRA :

Mass spectra was run on a GC-MS-QP5050A. GC-17A mass spectrophotometer at BCSIR, Dhaka.

2.1.8. MINIMUM EFFORT COLUMN CHROMATOGRAPHY :

The method of minimum effort column chromatography was used for fractionation of crude mixtures. The column was made by packing dry silica gel as the stationary phase (kiesel gel 60, 230-400 mesh, ASTM, MERK) in a heavy walled glass column equipped with air tight connectors of both sides and the crude absorbed in sillca gel was placed on the top of the column. The solvent as eluent was pumped on the top of the column by a FMI pump and the eluents were collected' at regular intervals.

2.1.9 PREPARATION OF REAGENTS INCLUDING SPRAY REAGENTS FOR CHROMATOGRAM.

Draggendorff reagent :

Bismuth nitrate (1.7 ml) was dissolved in distilled water (80ml) and acetic acid (20 ml) was then added to give solution A. Potassium iodide (32 g) was dissolved in distilled water (80 ml) to give solution B. The two solutions (solution A and solution B), 10 ml of each were mixed with distilled water (20 ml) and acetic acid (4 ml) to give the reagent.

Mayer's reagent :

Mercuric chloride (1.4 g) was dissolved in distilled water (60 ml) and was poured into a solution of potassium iodide (5 g) in distilled water (10 ml). The volume of the solution was made 100 ml by adding required amount of water to give mayer's reagent.

Spraying reagents :

Spraying reagents were prepared by dissolving 0.5g of vaniline in 6 ml concentrated sulphuric acid followed by dilution with 100 ml absolute ethanol.

Aniline-diphenyl amine-phosphoric acid reagent :

2 g of diphenyl amine, 2 ml aniline and 10 ml 85% phosphoric acid were dissolved in 100 ml of acetone to give aniline-diphenyl amine-phosphoric acid reagent.

2.1.10. COLLECTION AND PREPARATION OF SAMPLES FOR EXTRACTION :

The leaves and Twigs were collected from Barisal and Bangladesh University of Engineering & Technology Campus, Dhaka. The samples were sliced in small pices & well dried at room temperature. The total weight of dried simple 503. 598g. Dried sample of *"Vitis quadrangmlaris"* was divided into two parts and each part was extracted with pet. ether, chloroform and methanol at room temperature as per scheme in Fig : (11).

2.1.11. EXTRACTION OF MELHANOLIC EXTRACT :

The dried sample of *V. quadrangularis* (251.78 gm) was soaked in methanol and extracted successively at room temperature for 7 days. The solvents were rmoved from the extracts on a rotary vacuum evaporator under a reduced pressure at temperature below 40-45°C to yield a deep greenish gummy mass (16.16 gm), the extract was denoted as 'M'.

1.1.12. EXAMINATION OF EXTRACT-M :

The extract M (methanol extract) of *Vitis quadrangularis* was green gummy mass. It was almost insoluble in pet ether, acetone, ethylacetate but highly souble in methanol and chloroform. TLC examination of the extract was carried out in pet. ether, ethyl acetate in methanol and mixture of solvents to obtain good resolution. The resolution of fraction of TLC plates was best in ethylacetate : Pet ether, 1:5 which exibited three distinct spots with R_f . values 0.956, 0.882, 0.779 respectively with a tailing from the base line.

1.1.13. MINIMUM EFFORT COLUMN CHROMATORGAPIC SEPARATION OF FRACTION "M"

Fraction M (10.0g) was dissolved in a minimum volume of methanol and adsorbed in a small quantity of silica gel.The adsorbed mass was completely dried under reduced pressure and carefully poured on to the top of a column of silica gel of a minimum effort column chromatographic unit. The column was then eluted with pet. ether, mixtures of pet. ether-ethyl-acetate,ethylacetate-methanol and finally washed with methanol. A number of coloured bands e.g.; faint yellow and brown were observed during the development of the column. Fractions of about 12-15 ml were collected in EAC test tube at regular intervals and checked on TLC plates. In all 85 collections were made. The detailed results of the chromatograhic separation are shown in Table (1). Collections showing similar or almost similar TLC behaviour were combined together and the total collections were combined to obtain three, fracions, M_1.-M_3.

2.1.14. PREPARATION OF TLC PLATES :

Unless otherwise specified in the monograph, the plates are prepared in the following manner. A suspension of the coating substance was prepared in accoradnce with the instructions of the supplier and, using a spreading device designed for the-purpose, spread a uniform layer of the suspension 0.25 to 0.30 mm thick on a flat glass plate 20 cm long. Allow the coated plates to dry in air, heat at 100° C to 105° C for at least one hour the prepard plates were protected from moisture. Store the plates & protected from moisture and use with in three days of preparation. At the time of use, re-dried the plates.

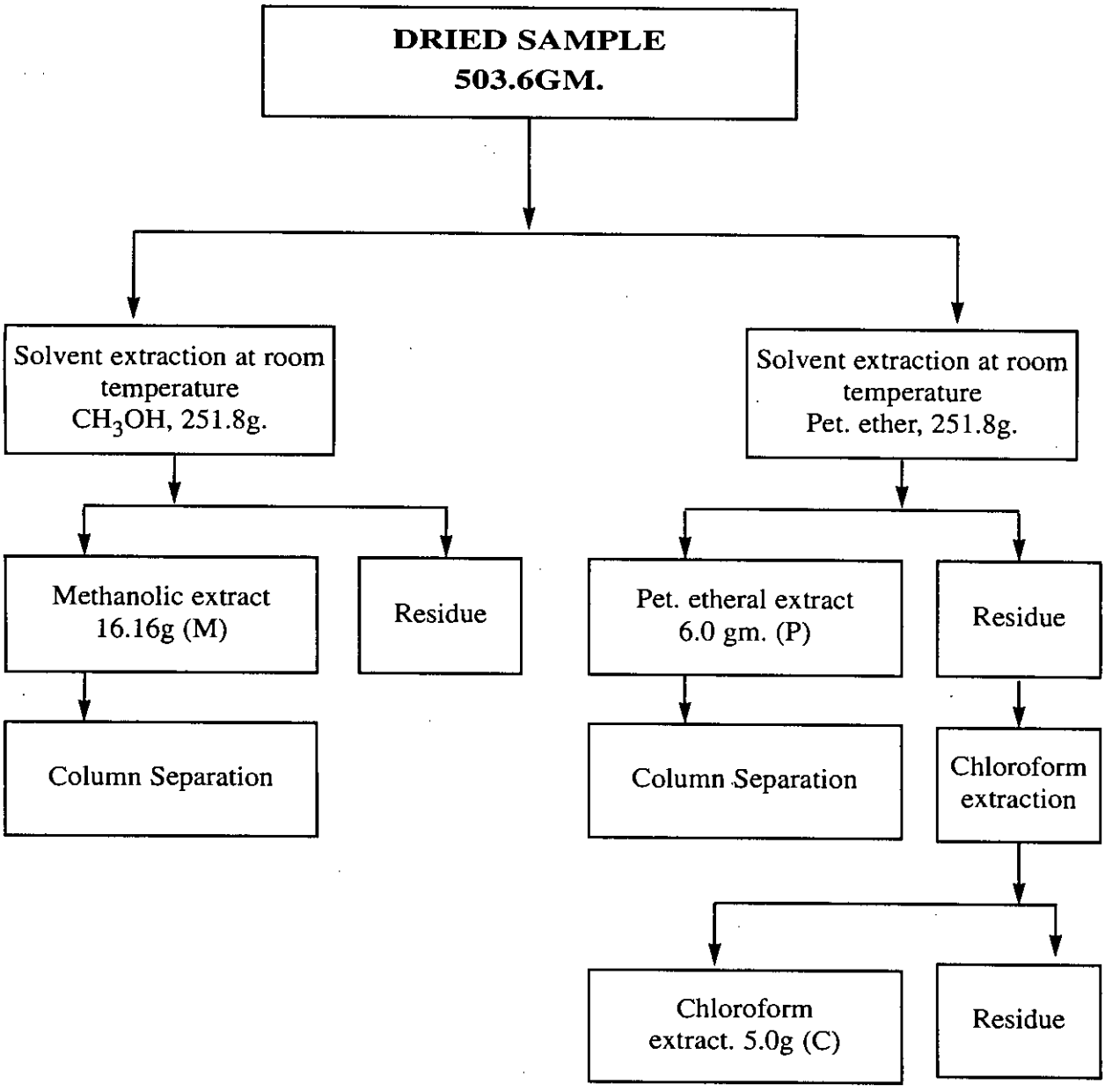


FIG. (11) EXTRACTION SCHEME OF *VITIS QUADRANGULARIS*

Table - 1

MINIMUM EFFORT COLUMN CHROMATOGRAPHIC SEPERATION OF FRACTION 'M' (METHANOLIC EXTRACT) :

[Eluating solvent ; Pet. ether, gradient mixtures of Pet. ether-ethylacetate, ethylacetate-melhanol, methanol]

Collection nos.	TLC Examination	Behaviour with Venilin-Sulfuric acid Reagent	Yield and Observation.
1 - 34	no Spot	–	–
35 - 59	(EA : PE, 1:4) Single Spot with tailing R_f 0.792	Violet coloration	1.5gm of Crude Product Fraction M_1.
60 - 70	Two Spots R_f 0.59, 0.52 (EA : PE, 1: 5)	Violet coloration	Fraction M_2 0.2 gm.
71 - 80	3 Spots R_f 0.32, 0.29, 0.25 (EA : ME, 1 : 4)	No coloration	Fraction M_3 0.1 gm.
81 - 85	No Spot	–	–

2.1.15. SAMPLE APPLICATION : (SPOTTING THE PLATES) :

A TLC plate is spotted with a small amount of the extracts or column eluates usually by using a fine glass capillary tube.

2.1.16 : PREPARATION OF TLC TANKS AND DEVELOPMENT OF CHROMATOGRAM :

Required amount of a suitable solvent system is poured into a chromatographic glass tank. The tank is then covered with a lid and kept for a certain period for attinment of saturation. A filter paper is usually introduced into the tank in order to promote the process of saturation. A spotted TLC plate is then placed in the satureated tank so that the solvent system applied at the bottom of the tank remains below the point of spot application in the plate. The chromatogram is then developed in an ascending sanner. During development as the solvent rises upward, the plate becomes graduallly moistened. Adequate care must be taken so that the solvent front does not travel beyond the upper end of the sillica coated surface of the TLC plate. As soon as the solvent front rises almost near the upper end of the silica coated plate, it is taken out and dried in an oven at 105°C (Donald et al., 1976) for visualization by using various spray reagents or in an iodine chamber.

2.1.17. DETECTION OF COMPOUNDS ON THE DEVELOPED CHROMATOGRAM :

The developed chromatoplates are dried at room temperature by hot air blow from a hair drier and the compound / compounds on the plates are located by using any one of the follwoing methods :

(A) UV-light : The compounds on the developed and dried TLC plates are viewed under UV-light at 254 nm and 366 nm. Some of the compunds appeared as fluorescing while others as dark spots under UV-light.

(B) Iodine vapour is a very common and versatile reagent for locating compounds in developed TLC plates.

(C) Vanillin-sulphuric acid spray : The developed plates are sprayed with vanilin-sulphuric acid reagnt and then heated at 110°C for 10 minutes. The resolvd compunds were identified with the development of specific colour (Mathews, 1963).

(D) DRAGENDROFF'S EAGENT : The presence of an alkaloid is detected by the appearance of an orange-red spot on sprying the developed plate with a Dragendroff's reagent.

2.1.18. THE R_f VALUE : R_f value is defined as the ratio of the distance travelled by a substance and the distance travelled by the solvent (Figur 11-a)

$$R_f = \frac{\text{Distance travelled by a substance}}{\text{Distance travelled by a solvent}}$$

R_f value in a solvent system is a constant for any compound and it is a physical property of that compound (Donald, 1976)

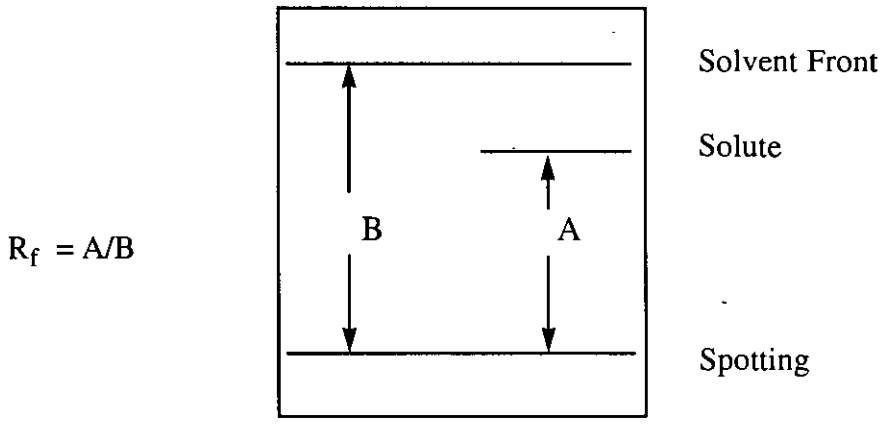


Fig. 11.(a) TLC plate for calculation of R_f value.

2.1.19. PURIFICATION OF THE FRACTION M_1 :

The fraction M_1 (1.5 gm) gave a single spot with tailing on TLC and was purified by washing repeatedly with pet. ether to remove adhering impurities. After wasing, a white residue was obtained. The residue was recrystallized from a mixture of solvent (EA : PE, 1:4) to give a pure compound (0.92 gm). This compound was designated S_1.

2.1.20. EXAMINATION OF FRACTION S_1 :

White crystalline substance(S_1) was is obtained from fraction M_1. In thin layer chromatography it shows one spot in (EA : PE, 1 : 4). The product is insoluble in pet. ether, ethylacetate, acetone but completely soluble in chlorofrom and methanol. Further recrystallization from a mixture of pet. ether and methanol gave a pure compound S_1 with a melting point of 240^0-243^0C.

2.1.21. PROPERTIES OF THE ISOLATED COMPOUND S_1 :

Physical appearance : White needle shaped crystalline substance.

Solubility : Insoluble in methanol, pet ether, acetone but completely soluble in chloroform.

Melting Point : 240-243°C

R_f Value : 0.792 (over silica gel, EtOAC : Pet ether, 1:4 as the mobile phase).

IR SPECTRUM :

The infrared spectra (Fig 12, 13, 14) of the compound S_1 run as KBr pellets showed characteristic absorbances at 2915 cm^{-1}, 1725 cm^{-1}, 1450 cm^{-1} and 1370 cm^{-1} due to C-H Stretching vibration and C=O stretching vibration respectively. The absorbance at 1245 cm^{-1} was due to C-O stretcing vibration. The absorbance at 720 cm^{-1} (with in a ring or in an open chain) due to methylene group.

^{1}H-NMR (400 MHz, $CDCl_3$) :

The ^{1}H-NMR (400 MHz) spectrum of S_1 (Fig 16,17,18) shoded signals at 0.73 (singlet), 0.87 (doublet), 0.89 (singlet), 1.01 (singlet), 1.00 (Singlet), 1.18 (Singlet), 1.05 (Singlet), 0.96 (Singlet), 1.96 (Multiplet), 2.28 (Multiplet) and 2.38 (Multiplet) ppm. and rest of the protons are about 22.

^{13}C - NMR SPECTROSCOPY (400) MHz) :

The ^{13}C- NMR (400 MHz) spectrum of compound S_1 (Fig 19,20,21,22) showed 30 signals and these were at 22.27, 41.51, 213.16, 58.22, 42.14, 42.29, 18.23, 53.10, 37.44, 59.48, 35.62, 30.49, 39.69, 28.29, 32.42, 36.01, 29.99, 42.80, 35.34, 28.16, 32.77, 39.07, 35.00 and 31.77 PPM.

GC-MASS SPECTROSCOPY :

The mass spectrum of compound S_1 (Fig 29,30) showed a molecular ion peak at m/z 426 indicating 426 as the molecular mass of the compound. The base peak of compound S_1 was at m/z 69 and other more intense peaks were at m/z 302, 246, 205, 218, 149, 179, 123, 95, 55 and 41.

2.1.22 EXTRACTION OF PET. ETHER EXTRACT AND CHLOROFROM EXTRACT :

The dried sample of *V. quadrangularis* (251.78 gm) was dissolved in pet. ether (b.p. 60°C-80°C) and extracted successively for 7 days. Then filtered the extract and the residue was again dissoved in chloroform and extracted successively. The solvents were removed from the extracts on a rotary vacuum evaporator under a reduced pressure and a temperature below 40-45°C to yield brown gummy mass (6.0 gm) from pet. ether and labelled as 'P' and also mre green gummy mass (5.0 gm) was obtained from chloroform and named as 'C'.

2.1.23. EXAMINATION OF EXTRACT "P" :

The extract P (Pet. ether extract) of *"Vitis quadrangularis"* was brownish gummy mass. It was insoluble in (CH_3-OH), acetone and completely soluble in pet. ether, ethyl-acetate and chloroform. TLC examination of this extract was carried out in, ethylacetate and pet. ether combinations and obtained good resolution. The resolution on TLC plates was.

best in 1:4, EA : PE. which exibite two spots at R_f 0.789 and 0.462 respectively with tailing from the base line.

2.1.24. MINIMUM EFFORT COLUMN CHROMATOGRAPHIC SEPARATION OF FRACTION "P" :

Fraction P (5.5 gm) was dissolved in a minimum of Pet. ether and adsorbed in small quantity of silica gel. The adsorbed mass was completely dried under reduced pressure and carefully poured on the top of a column of silica gel of a minimum effort column chromatographic unit.

The column was then eluted with pet. either, mixtures of pet. ether, ethyl-acetate, ethylacetate-methanol and finally washed with methanol. A number of colored bands e.g., faint yellow and brown were observed during the development of the column. Fractions of about 12-15 ml were collected in each test tube at regular intervals and checked on TLC plates. In all 105 collections were made. The detailed results of the chromatographe eperations are shown in table (2), collections showing similar or almost similar TLC behaviour were combined to gether and the total collections were combined to obtained two fraction, P_1 and P_2.

2.1.25. PURIFICATION OF THE FRACTION P_1 :

The fraction P_1 (1.5 gm) gave a single spot with tailing on TLC and was purified by washing repeatedly with pet. ether to remove associated impurities. After wasing a white residue was obtained. The residue wasre crystallized from PE : EA, 1:5 crystals were dried and a pure compound (0.78 gm) was obtained. This compound was designated as S_2.

2.1.26. EXAMINATION OF THE FRACTION S_2 :

White crystalline substance(S_2). This is obtained from fraction P_1. In thin layer chroatography it shows one spot in (PE : EA, 1 : 5). The product is insoluble in pet. ether, ethylacetate, acetone but completely soluble in chlorofrom and methanol. Further recrystallization from a mixture of pet. ether and methanol gave pure compound S_2 with a melting point of 240^0-242^0C.

Table - 2

MINIMUM EFFORT COLUMN CHROMATOGRAPHIC SEPERATION OF FRACTION 'P' (PET. ETHER EXTRACT) :

[Eluting solvent, Pet. ether, gradient mixtures of Pet.ether- ethylacetate, ethylacetate - methanol, methanol].

Collection nos.	TLC Examination	Behaviour with Venilin-Sulfuric acid Reagent	Yield and Observation.
1 - 19	no spot	–	–
20 - 55	Single spot with tailing R_f 0.789 (PE : EA, 1 : 5)	Violet coloration	1 . 4 gm. of Crude product. fraction P_1.
55 - 85	Two spot with tailing R_f 0.503, 0.462 (EA : PE, 1:4)	No Coloration	0.05 gm. of two mixtures fraction P_2
85 - 105	no spot	–	–

2.1.27. PROPERTIES OF THE ISOLATED COMPOND S_2 :

Physical appearance : White coloured crystalline substance.

Solubility : Insoluble in methanol, slighty soluble in ethyl acetate with 107°C heat, insoluble in pet ether, completely soluble in chloroform.

Melting Point : 240-242°C

R_f value : 0.789 (over silica gell, pet. ether : EtOAC, 1:5 as the mobile phase).

IR SPECTRUM : The infrared spectra (Fig 15) of the compound S_2 run as KBr pellets showed characteristic absorbances at 2915 cm^{-1}, 1725 cm^{-1} and at 1350 cm^{-1} due to C-H Stretching vibration and C=O stretching vibration respectively. The absorbance at 1245 cm^{-1} was due to C-O stretcing vibration. The absorbance at 782 cm^{-1} (with in a ring or in an open chain) due to methylene group.

^{1}H-NMR (400 MHz, $CDCl_3$) : The 1H-NMR (400 MHz) spectrum of S_2 (Fig 23,24,25) showed signals at 0.73 (singlet), 0.87 (doublet), 0.89 (singlet), 1.01 (singlet), 1.00 (Singlet), 1.18 (Singlet), 1.05 (Singlet), 0.96 (Singlet), 1.96 (Multiplet), 2.28 (Multiplet) and 2.38 (Multiplet) ppm and rest of the protons are about 22.

^{13}C - NMR SPECTROSCOPY (400) MHz) :

The ^{13}C- NMR (400 MHz) spectrum of compound S_2 (Fig 26, 27,28) showed 30 signals and these were at 22.27, 41.51,

213.16, 58.22, 42.14, 42.29, 18.23, 53.10, 37.44, 59.48, 35.62, 30.49, 39.69, 28.29, 32.42, 36.01, 29.99, 42.80, 35.34, 28.16, 32.77, 39.07, 35.00 and 31.77 ppm.

GC-MASS SPECTROSCOPY :

The mass spectrum of compound S_2 (Fig 31,32) showed a molecular ion peak at m/z 426 indicating 426 as the molecular mass of the compound. The base peak of compound S_2 was at m/z 69 and other more intemse peaks were at m/z 302, 246, 205, 218, 149, 179, 123, 95, 55 and 41.

Table -3

MINIMUM EFFORT COLUMN CHROMATOGRAPHIC SEPERATION OF FRACTION C (CHLOROFORM EXTRACT) :

[Elating solvent ; Pet. ether, gradient mixtures of Pet.ether- ethylacetate, ethylacetate - methanol, methanol].

Collection nos.	TLC Examination	Behaviour with Venilin-Sulfuric acid Reagent	Yield and Observation.
1 - 11	no spot	–	–
12 - 40	Two Spot. R_f 0.864, 0.906 (PE : EA, 5 : 1)	Violet coloration	0.20 gm. of mixt. product. fraction C_1
40 - 70	Tne Spot. R_f 0.562, 0.482 (PE : EA, 5 : 2)	No Coloration	0.102 gm. of combined product fraction C_2
70 - 85	no Spot.	–	–

2.1.28. EXAMINATION OF EXTRACT-"C" :

The extract C (Chloroform extract) of *"Vitis quadrangularis"* was deep green gummy mass. It was insouble in pet ethar, acetone and melhanol but completely soluble in ethylacetate and chlorofom. TLC examination of this extract was carried out in pet. ethar, ethylacetate and methanol and their combinations for obtaining good resolution. The resolution on TLC plates was best in 5 : 1, PE : EA in which exibites four spots at R_f 0.906, 0.864, 0.562, 0.482 respectively with tailing from the base line.

2.1.29. MINIMUM EFFORT COLUMN CHROMATOGRAPHIC SEPARATION OF FRACTION 'C' :

Fraction C (2.5) gm.) was dissolved in chloroform and adsorbed in small quantity of silica gel. The adsorbed mass was completely dried under reduced pressure and carefully poured on the top of a column of silica gel of a minimum effort column chromatographic unit. The column was then eluted with pet. ether, mixtures of pet.ethar - ethylacetate, ethylacetate - methanol and finally washed with methanol. A number of coloured bands e.g., faint yellow, green gummy brown observed during the development of the column. Fractions of about 12-15 ml were collected in each test tube at regular intervals and checked on TLC plates. In all 85 collections were made. The detailed results of the chromatographic separations are shown in table (3) collections showing similar or almost similar TLC behaviour were combined together and the total collections were combined to obtain two fraction, C_1 & C_2 .

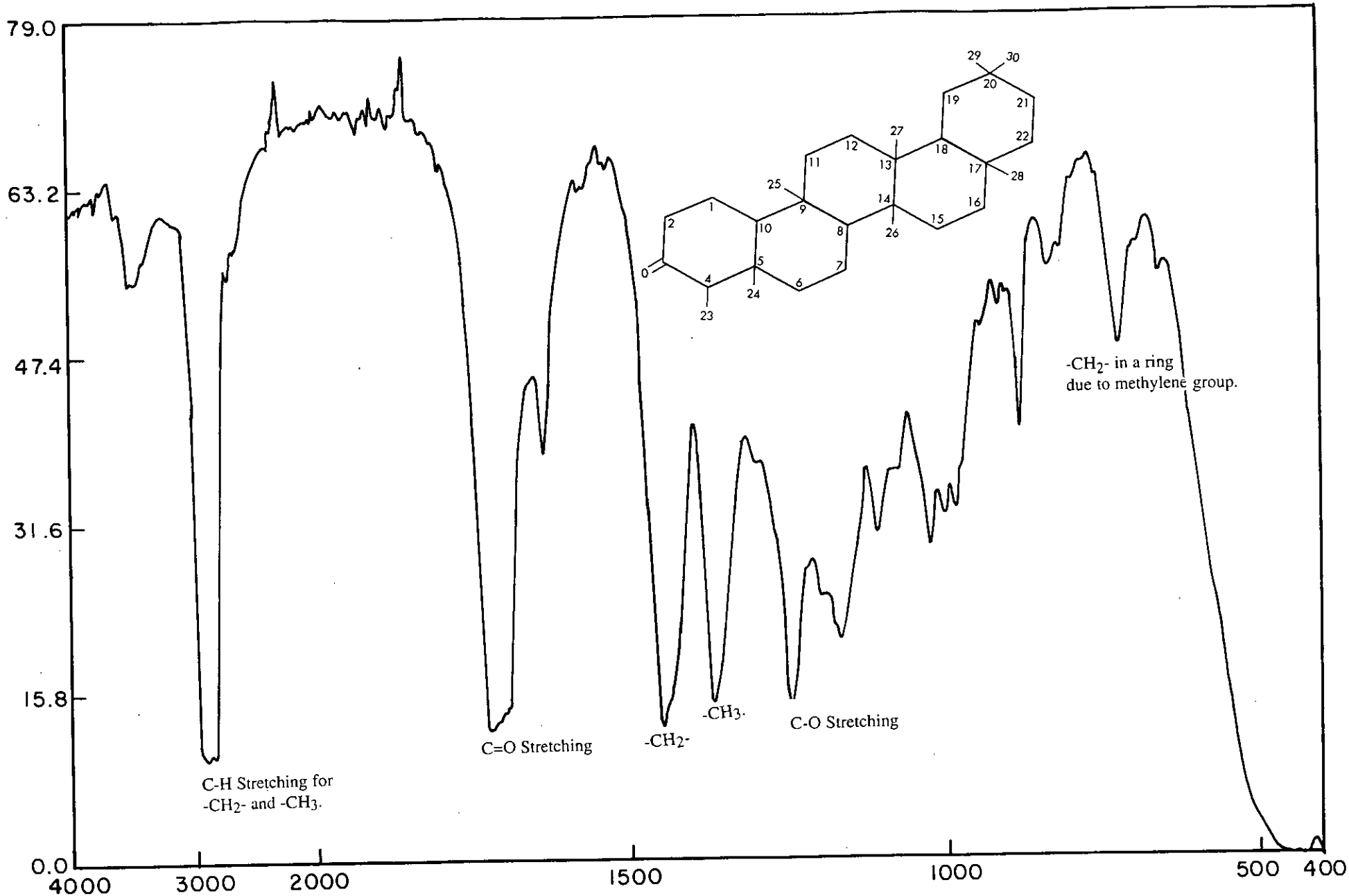


Fig. : (12) IR - Spectrum of Sample M_1

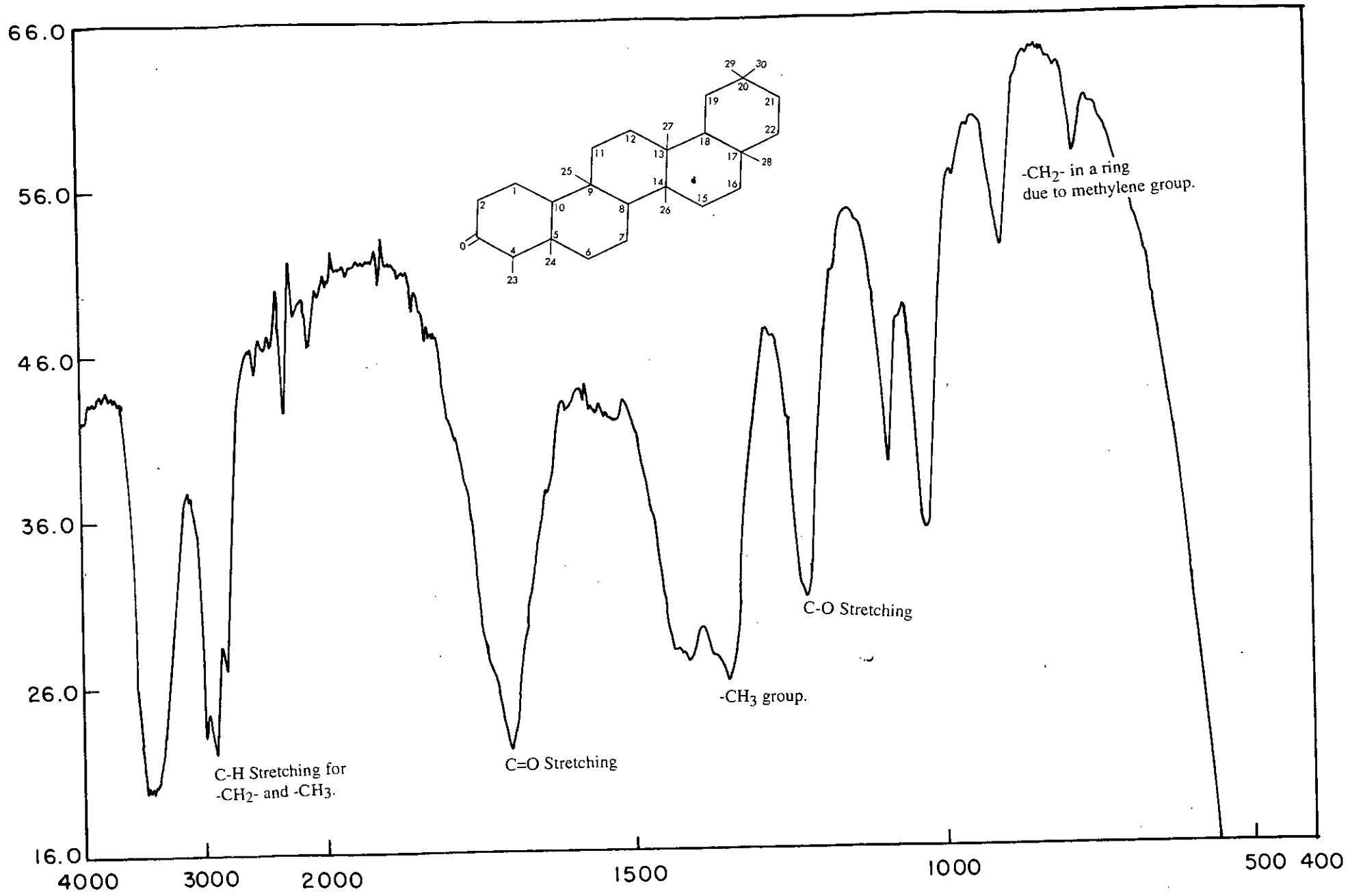


Fig. : (13) IR - Spectrum of Sample S_1

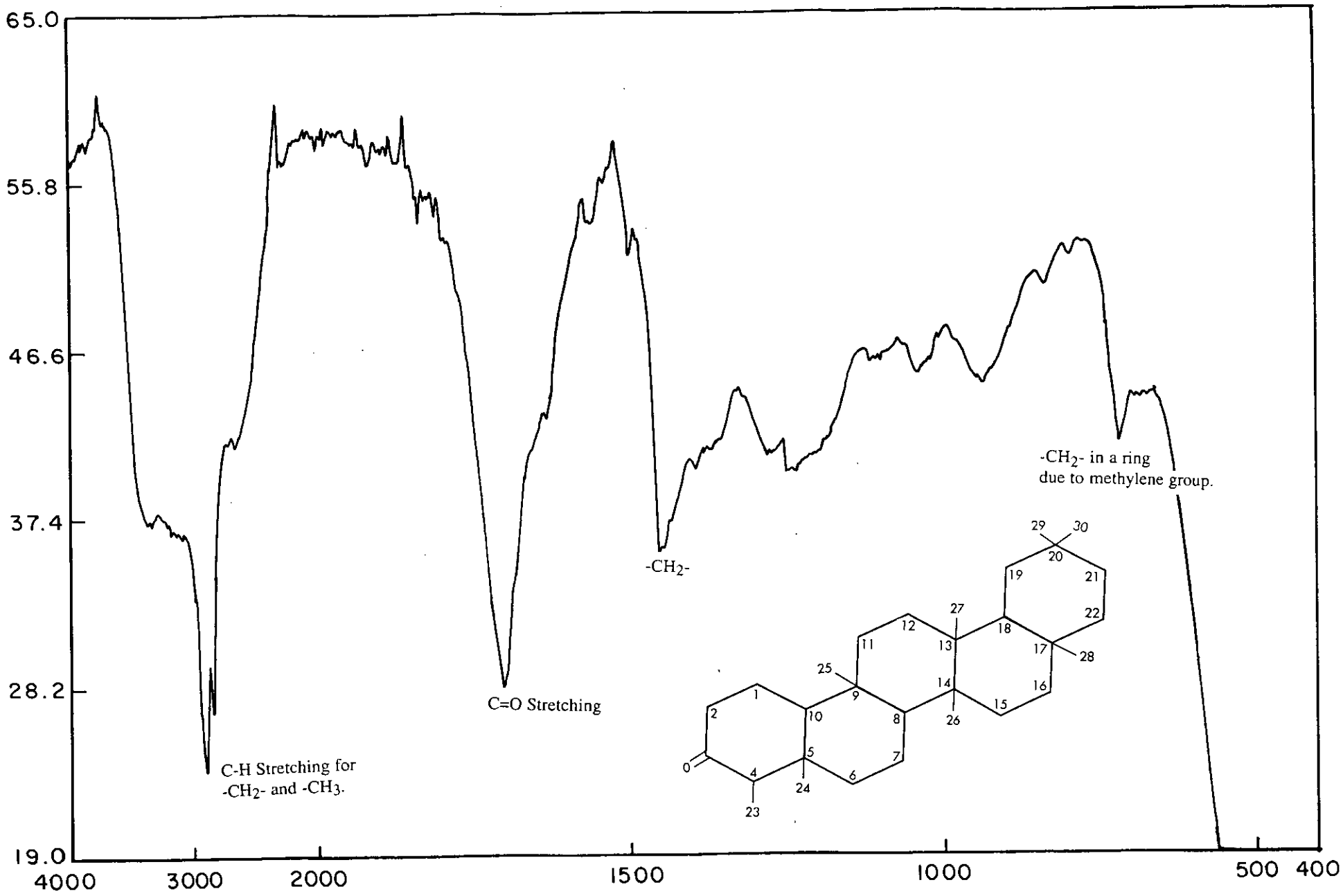


Fig. : (14) IR - Spectrum of Sample M_2

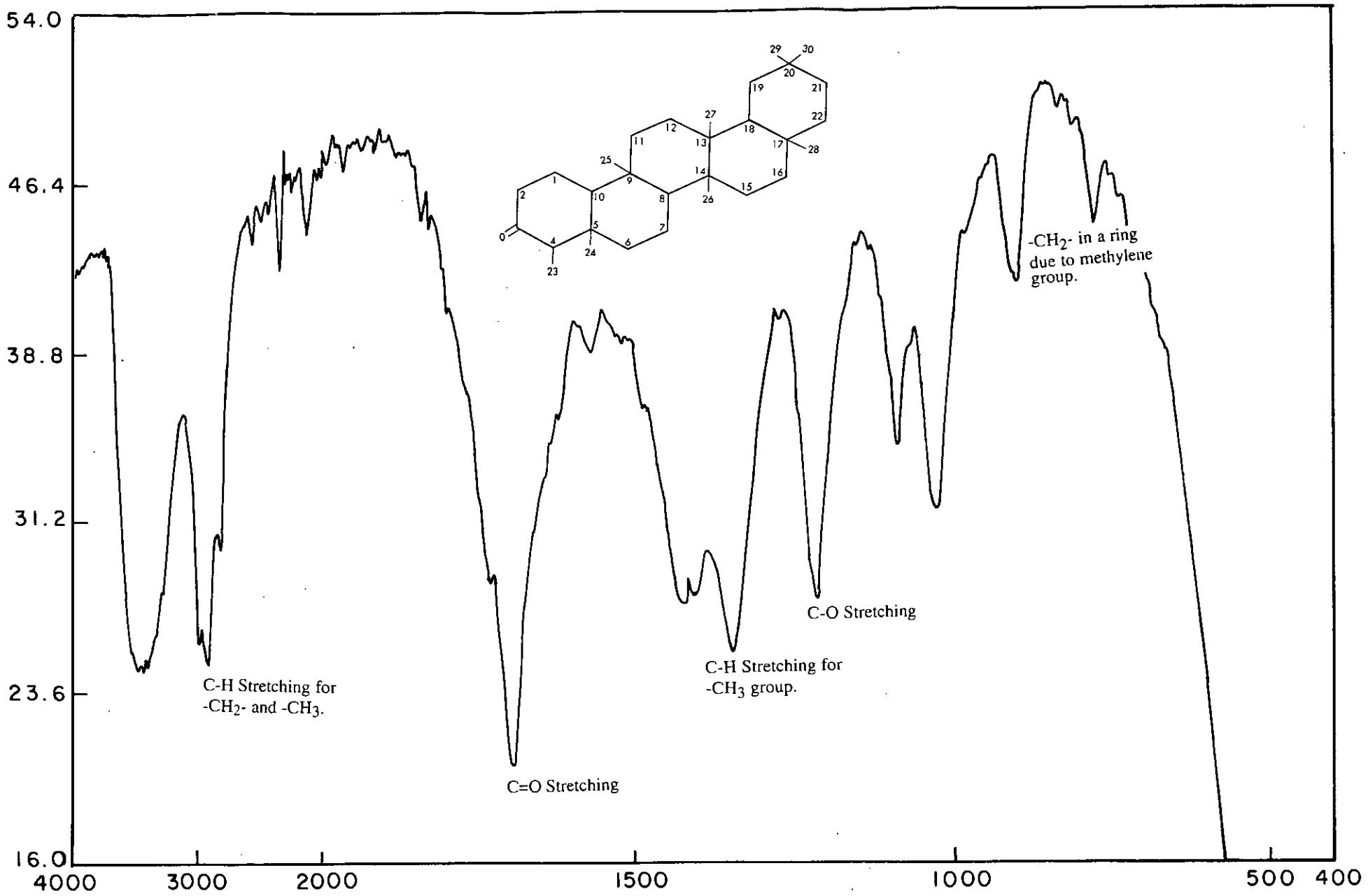
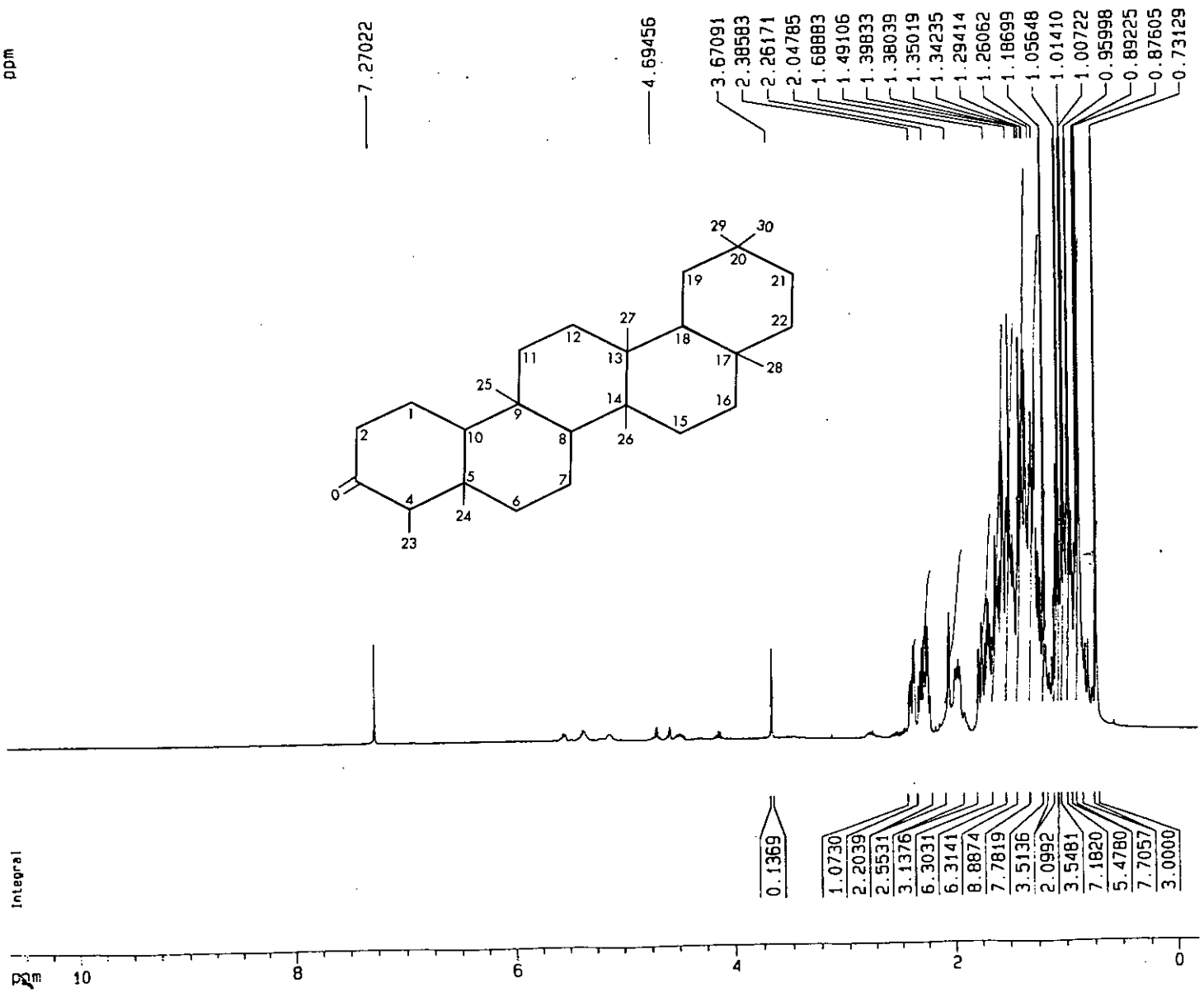


Fig. : (15) IR - Spectrum of Sample S_2

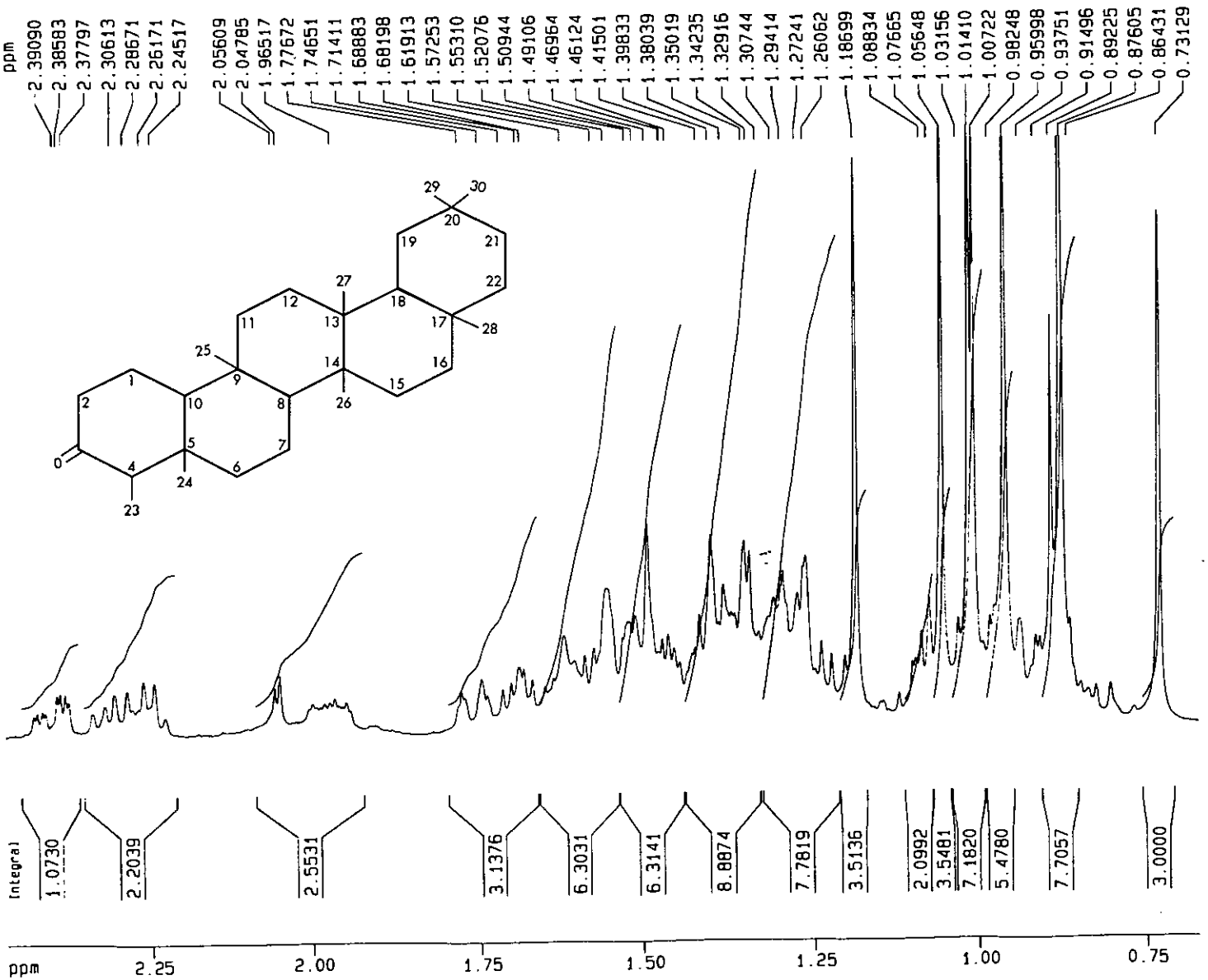
1H Spectrum S1 in CDCl3 (Moni, BUET)

Fig. : (16) ^{1}H Spectrum of Sample S_1 in $CDCl_3$



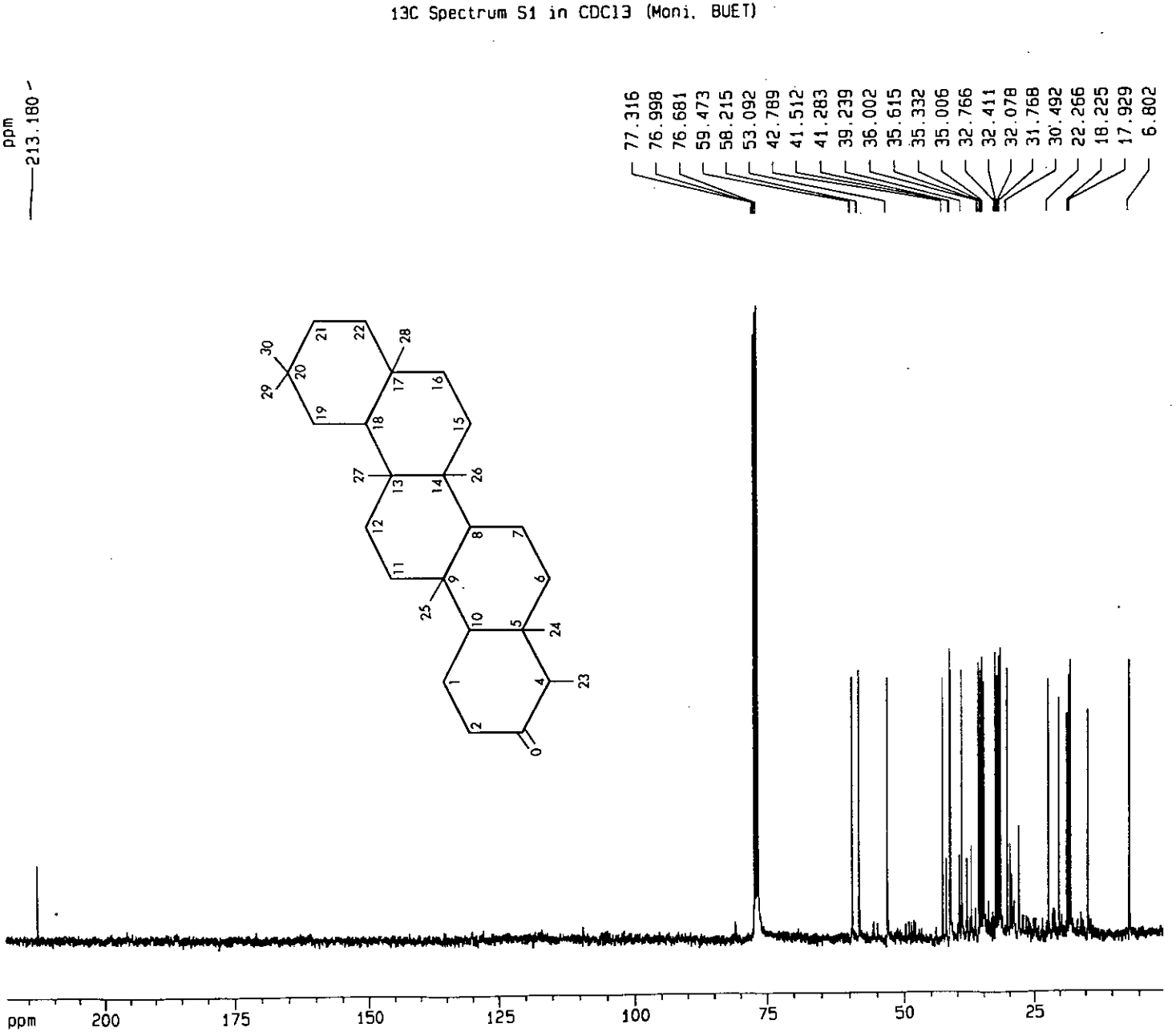

```
Current Data Parameters
NAME                  A119
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_              20010227
Time                 10.43
INSTRUM              dpx400
PROBHD     5 mm Multinuc
PULPROG                zg
TD                  32768
SOLVENT             CDCl3
NS                     32
OS                      0
SWH            4789.272 Hz
FIORES         0.146157 Hz
AQ             3.4210291 sec
RG                     64
DW              104.400 usec
DE                6.00 usec
TE                310.0 K
D1           1.00000000 sec

============ CHANNEL f1 =============
NUC1                   1H
P1                 8.30 usec
PL1                -6.00 dB
SFO1        400.1420007 MHz

F2 - Processing parameters
SI                  32768
SF          400.1400047 MHz
WDW                    EM
SSB                     0
LB                 0.30 Hz
GB                      0
PC                   1.40

1D NMR plot parameters
CX                20.00 cm
F1P              10.655 ppm
F1             4263.36 Hz
F2P              -0.187 ppm
F2               -74.75 Hz
PPMCM          0.54207 ppm/cm
HZCM         216.90543 Hz/cm
```

Fig. : (17) ^{1}H Spectrum of Sample S_1 in $CDCl_3$




```
Current Data Parameters
NAME                  A119
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_              20010227
Time                 10.43
INSTRUM              dpx400
PROBHD     5 mm Multinuc
PULPROG                 zg
TD                   32768
SOLVENT              COCl3
NS                      32
DS                       0
SWH              4789.272 Hz
FIDRES           0.146157 Hz
AQ               3.4210291 sec
RG                      64
DW                104.400 usec
DE                  6.00 usec
TE                 310.0 K
D1              1.00000000 sec

============ CHANNEL f1 =============
NUC1                   1H
P1                   8.30 usec
PL1                 -6.00 dB
SFO1           400.1420007 MHz

F2 - Processing parameters
SI                   32768
SF             400.1400047 MHz
WDW                    EM
SSB                     0
LB                   0.30 Hz
GB                      0
PC                   1.40

1D NMR plot parameters
CX                  20.00 cm
F1P                 2.468 ppm
F1                  987.57 Hz
F2P                 0.671 ppm
F2                  268.32 Hz
PPMCM            0.08987 ppm/cm
HZCM             35.96234 Hz/cm
```

Fig. : (18) ^{13}C Spectrum of Sample S_1 in $CDCl_3$



Current Data Parameters

NAME	A119
EXPNO	2
PROCNO	1

F2 - Acquisition Parameters

Date_	20010227
Time	14.08
INSTRUM	dpx400
PROBHD	5 mm Multinuc
PULPROG	zgpg
TD	32768
SOLVENT	CDCl3
NS	7000
DS	0
SWH	22075.055 Hz
FIDRES	0.673677 Hz
AQ	0.7422452 sec
RG	16384
DW	22.650 usec
DE	6.00 usec
TE	300.0 K
D1	1.50000000 sec
d11	0.03000000 sec
d12	0.00002000 sec

========== CHANNEL f1 ==========

NUC1	13C
P1	6.60 usec
PL1	-6.00 dB
SFO1	100.6263107 MHz

========== CHANNEL f2 ==========

CPDPRG2	waltz16
NUC2	1H
PCPD2	80.00 usec
PL2	-6.00 dB
PL12	16.00 dB
PL13	120.00 dB
SFO2	400.1400000 MHz

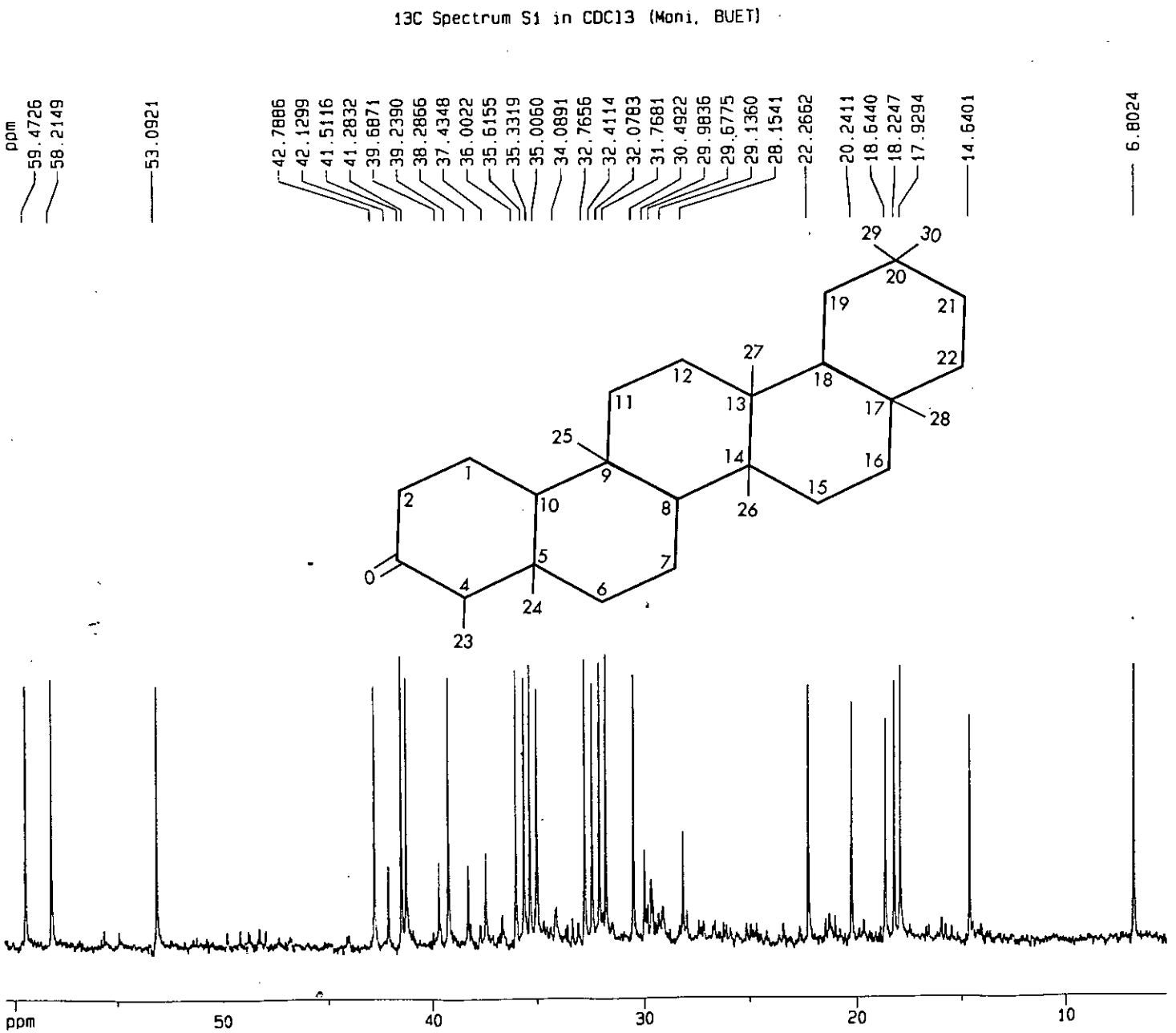
F2 - Processing parameters

SI	32768
SF	100.6152852 MHz
WDW	EM
SSB	0
LB	2.50 Hz
GB	0
PC	1.40

1D NMR plot parameters

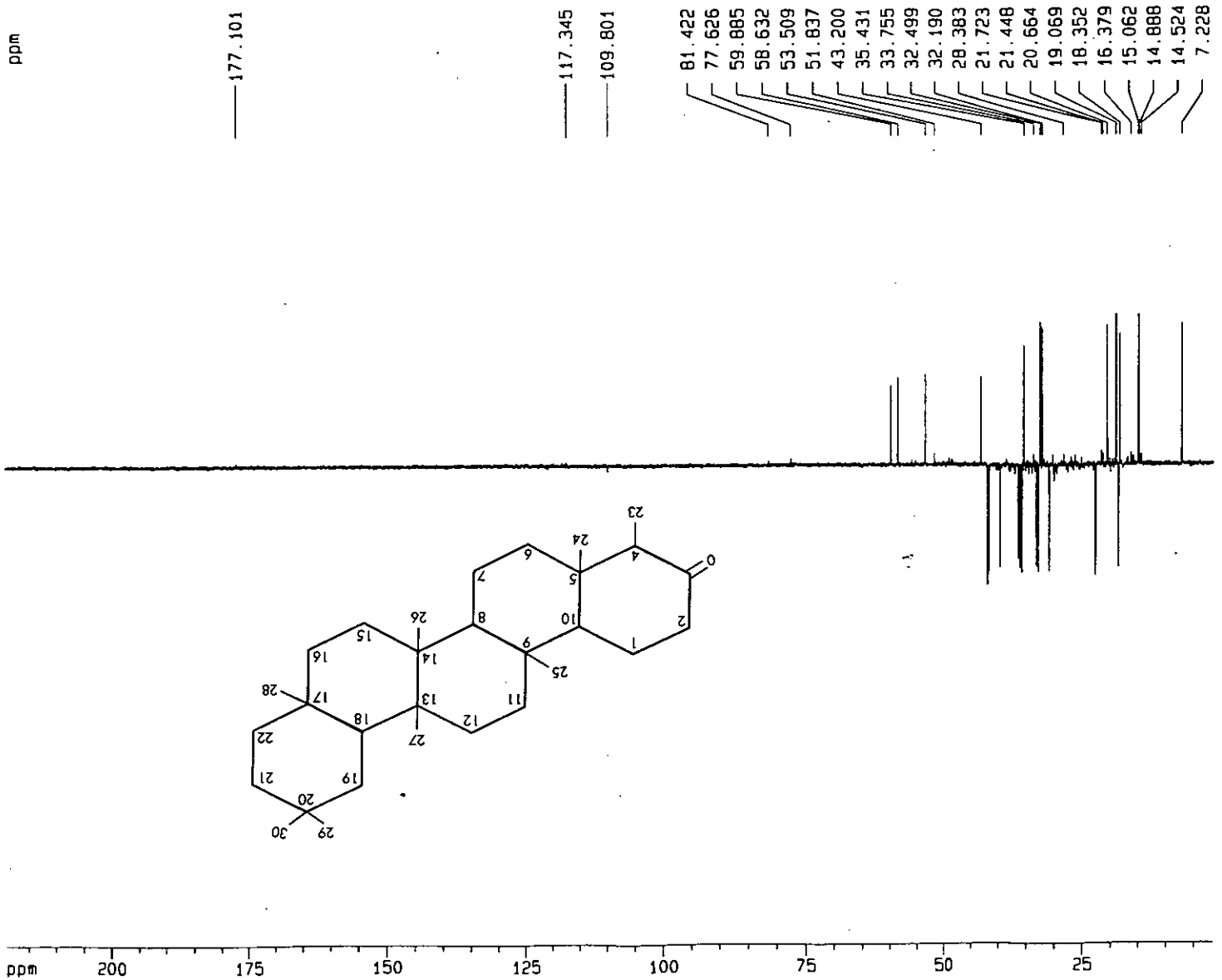
CX	20.00 cm
F1P	219.281 ppm
F1	22063.02 Hz
F2P	0.675 ppm
F2	67.95 Hz
PPMCM	10.93028 ppm/cm
HZCM	1099.75366 Hz/cm

Fig. : (19) ^{13}C Spectrum of Sample S_1 in $CDCl_3$



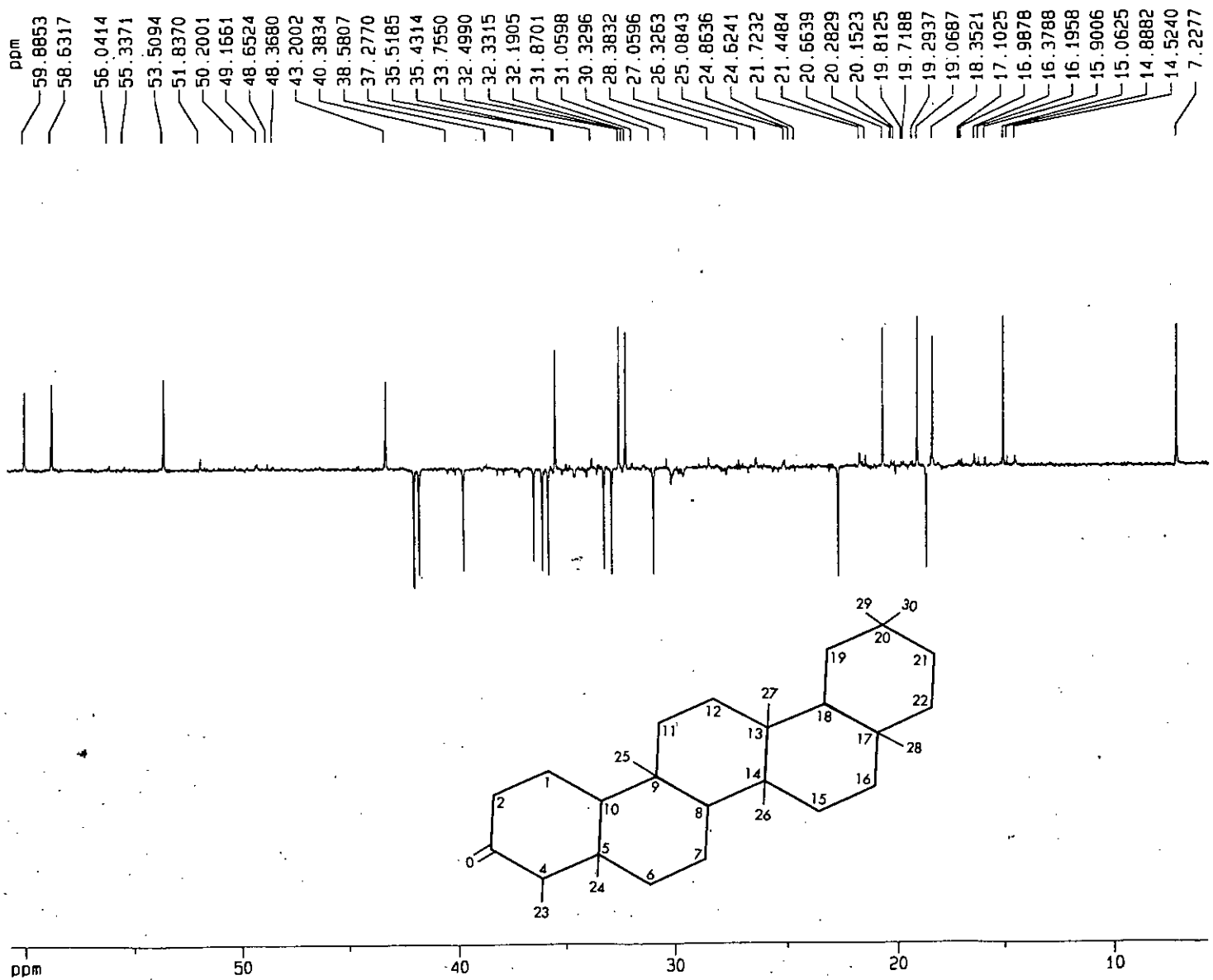

```
Current Data Parameters
NAME                 A119
EXPNO                   2
PROCNO                  1

F2 - Acquisition Parameters
Date_             20010227
Time                 14.08
INSTRUM              dpx400
PROBHD     5 mm Multinuc
PULPROG              zgpg
TD                  32768
SOLVENT              CDCl3
NS                    7000
DS                       0
SWH              22075.055 Hz
FIDRES           0.673677 Hz
AQ               0.7422452 sec
RG                   16384
DW                 22.650 usec
DE                    6.00 usec
TE                   300.0 K
D1              1.50000000 sec
d11             0.03000000 sec
d12             0.00002000 sec

========== CHANNEL f1 ==========
NUC1                  13C
P1                   6.60 usec
PL1                 -6.00 dB
SFO1          100.6263107 MHz

========== CHANNEL f2 ==========
CPDPRG2           waltz16
NUC2                  1H
PCPD2               80.00 usec
PL2                 -6.00 dB
PL12                16.00 dB
PL13               120.00 dB
SFO2          400.1400000 MHz

F2 - Processing parameters
SI                   32768
SF            100.6152852 MHz
WDW                    EM
SSB                     0
LB                   2.50 Hz
GB                      0
PC                   1.40

1D NMR plot parameters
CX                  20.00 cm
F1P                 60.466 ppm
F1                6083.85 Hz
F2P                  5.224 ppm
F2                 525.60 Hz
PPMCM             2.76213 ppm/cm
HZCM            277.91241 Hz/cm
```

Fig. : (20) Dept 135 of Sample S_1 in $CDCl_3$




```
Current Data Parameters
NAME                 A119
EXPNO                   3
PROCNO                  1

F2 - Acquisition Parameters
Date_             20010227
Time                23.32
INSTRUM           dpx400
PROBHD     5 mm Multinuc
PULPROG          dept135
TD                 32768
SOLVENT            CDCl3
NS                  6000
DS                     8
SWH           22075.055 Hz
FIDRES         0.673577 Hz
AQ             0.7422452 sec
RG                 13004
DW               22.650 usec
DE                6.00 usec
TE                300.0 K
CNST2         145.0000000
D1              4.00000000 sec
d2              0.00344828 sec
d12             0.00002000 sec
DELTA           0.00000764 sec

============ CHANNEL f1 =============
NUC1                 13C
P1                  6.00 usec
p2                 12.00 usec
PL1                -6.00 dB
SFO1          100.6263107 MHz

============ CHANNEL f2 =============
CPDPRG2          waltz16
NUC2                 1H
P3                 8.30 usec
p4                16.60 usec
PCPD2             80.00 usec
PL2                -6.00 dB
PL12               16.00 dB
SFO2          400.1420007 MHz

F2 - Processing parameters
SI                 32768
SF          100.6152430 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40

1D NMR plot parameters
CX                20.00 cm
F1P              219.170 ppm
F1              22051.88 Hz
F2P               1.360 ppm
F2              136.79 Hz
PPMCM           10.89054 ppm/cm
HZCM          1095.75452 Hz/cm
```

Fig. : (21) Dept 135 of Sample S_1 in $CDCl_3$




```
Current Data Parameters
NAME                  A119
EXPNO                    3
PROCNO                   1

F2 - Acquisition Parameters
Date_              20010227
Time                 23.32
INSTRUM              dpx400
PROBHD     5 mm Multinuc
PULPROG            dept135
TD                   32768
SOLVENT              CDCl3
NS                    6000
DS                       8
SWH            22075.055 Hz
FIDRES          0.673677 Hz
AQ              0.7422452 sec
RG                   13004
DW                22.650 usec
DE                 6.00 usec
TE                300.0 K
CNST2          145.0000000
D1               4.00000000 sec
d2               0.00344828 sec
d12              0.00002000 sec
DELTA            0.00000764 sec

========== CHANNEL f1 ============
NUC1                  13C
P1                 6.00 usec
p2                12.00 usec
PL1               -6.00 dB
SFO1          100.6263107 MHz

========== CHANNEL f2 ============
CPDPRG2           waltz16
NUC2                  1H
P3                 8.30 usec
p4                16.60 usec
PCPD2             80.00 usec
PL2               -6.00 dB
PL12              16.00 dB
SFO2          400.1420007 MHz

F2 - Processing parameters
SI                   32768
SF            100.6152430 MHz
WDW                    EM
SSB                     0
LB                   1.00 Hz
GB                      0
PC                   1.40

1D NMR plot parameters
CX                  20.00 cm
F1P                60.696 ppm
F1                6106.94 Hz
F2P                5.753 ppm
F2                 578.87 Hz
PPMCM             2.74713 ppm/cm
HZCM             276.40314 Hz/cm
```

Fig. : (22) Dept 90 of Sample S_1 in $CDCl_3$

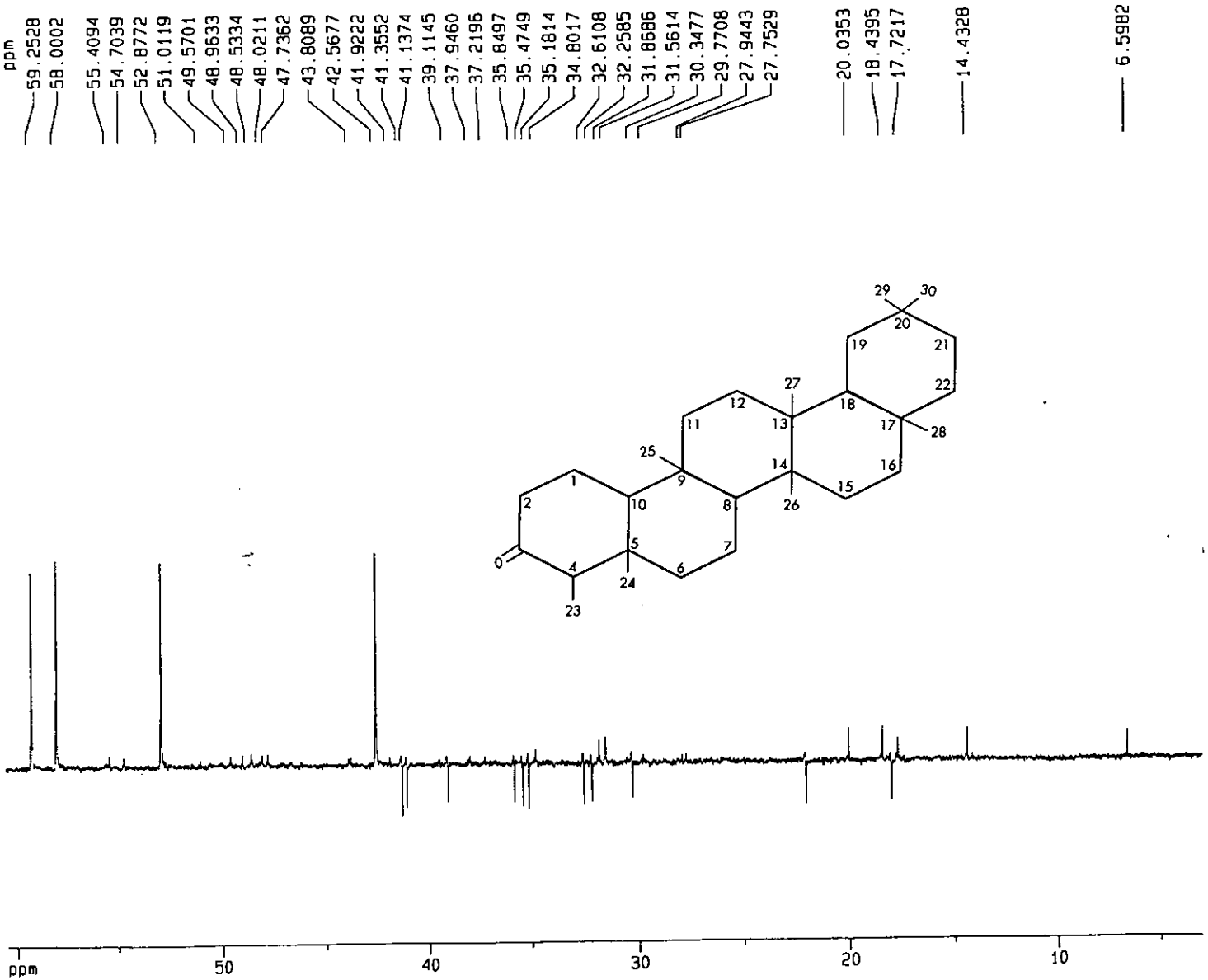
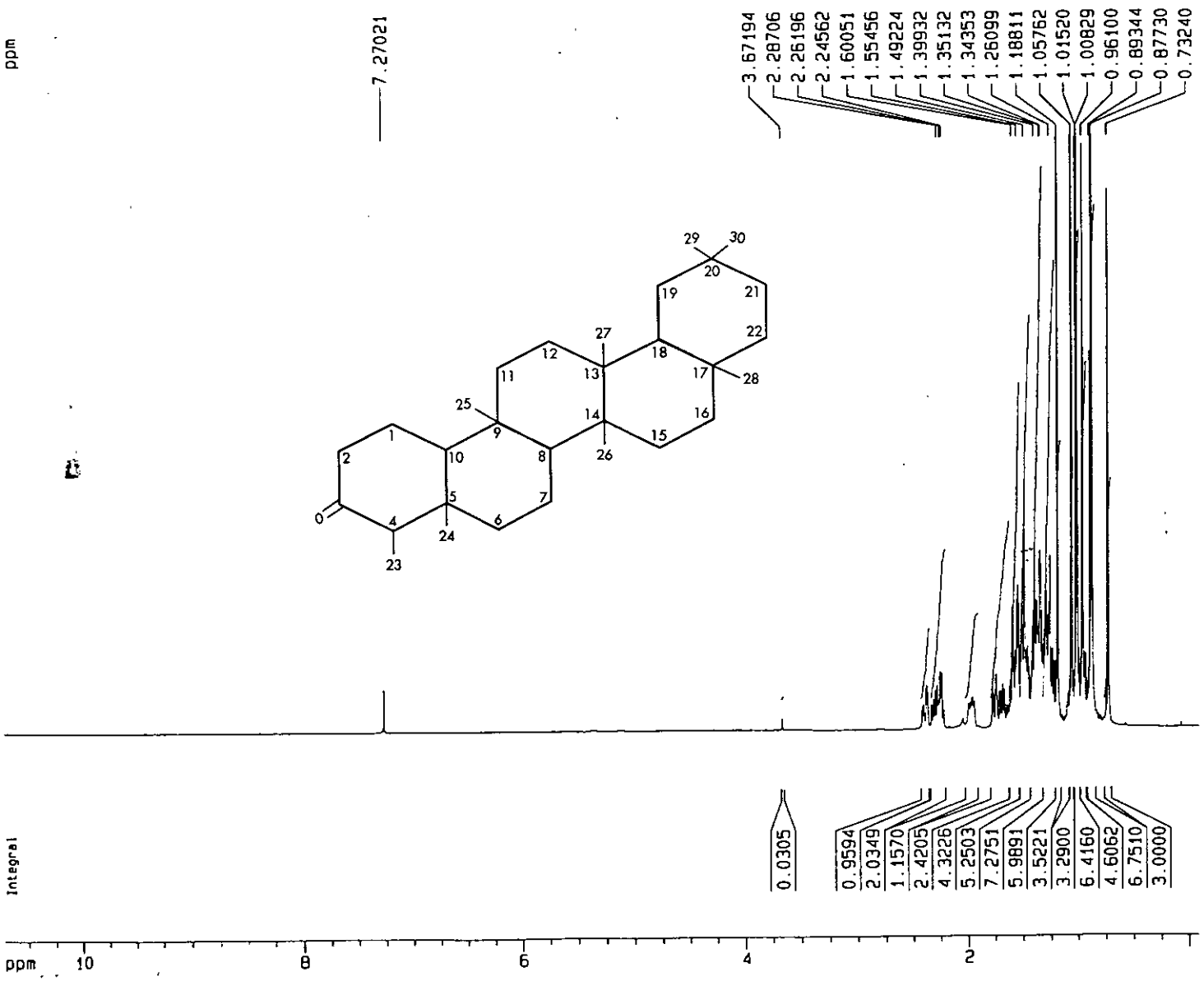


Fig. : (23) ^1H Spectrum of Sample S_2 in $CDCl_3$



Current Data Parameters

NAME	A120
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

Date_	20010228
Time	12.03
INSTRUM	dpx400
PROBHD	5 mm Multinuc
PULPROG	zg
TD	32768
SOLVENT	CDCl3
NS	32
DS	0
SWH	4789.272 Hz
FIDRES	0.146157 Hz
AQ	3.4210291 sec
RG	64
DW	104.400 usec
DE	6.00 usec
TE	310.0 K
D1	1.00000000 sec

========== CHANNEL f1 ==========

NUC1	1H
P1	8.30 usec
PL1	-6.00 dB
SFO1	400.1420007 MHz

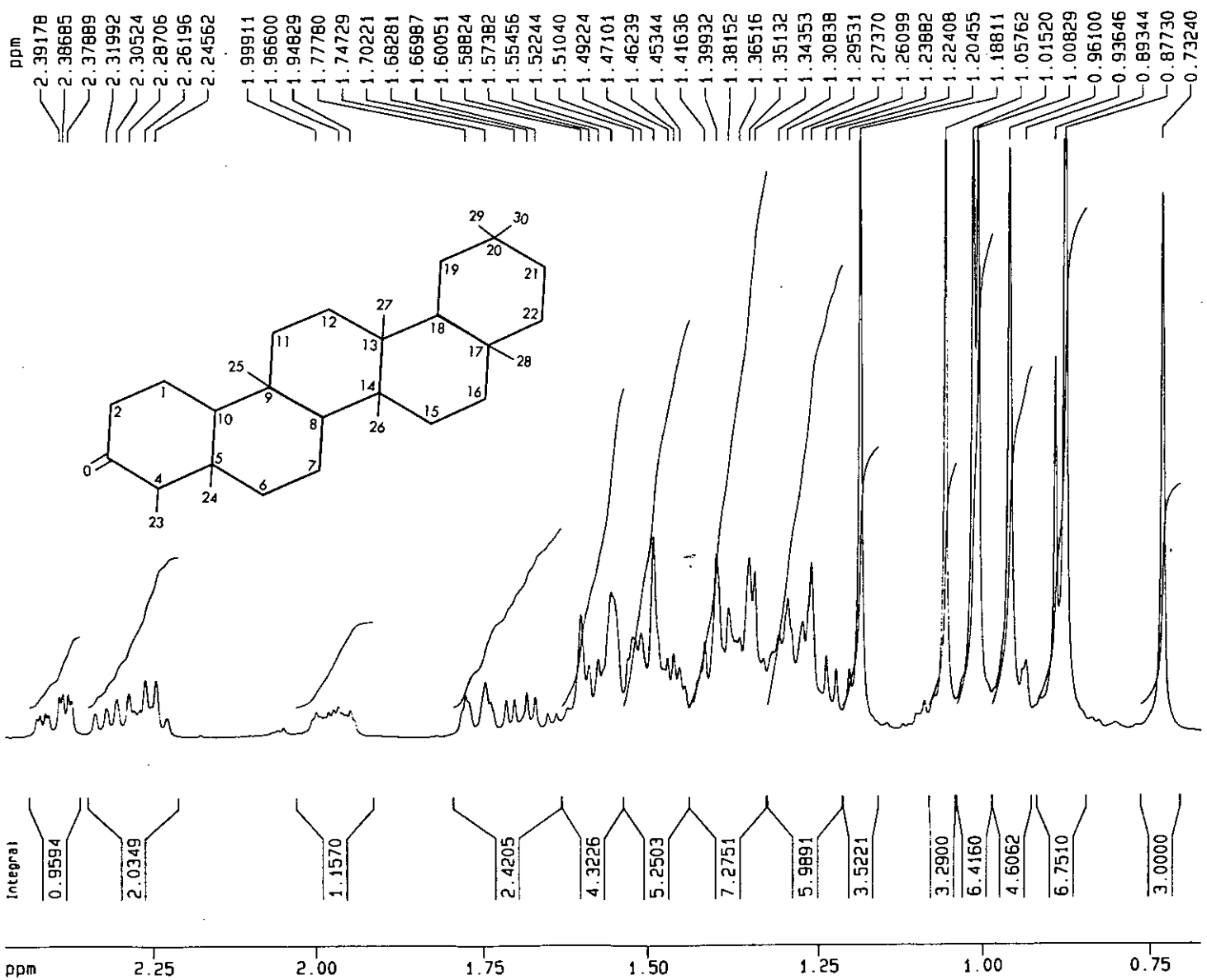
F2 - Processing parameters

SI	32768
SF	400.1400049 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.40

1D NMR plot parameters

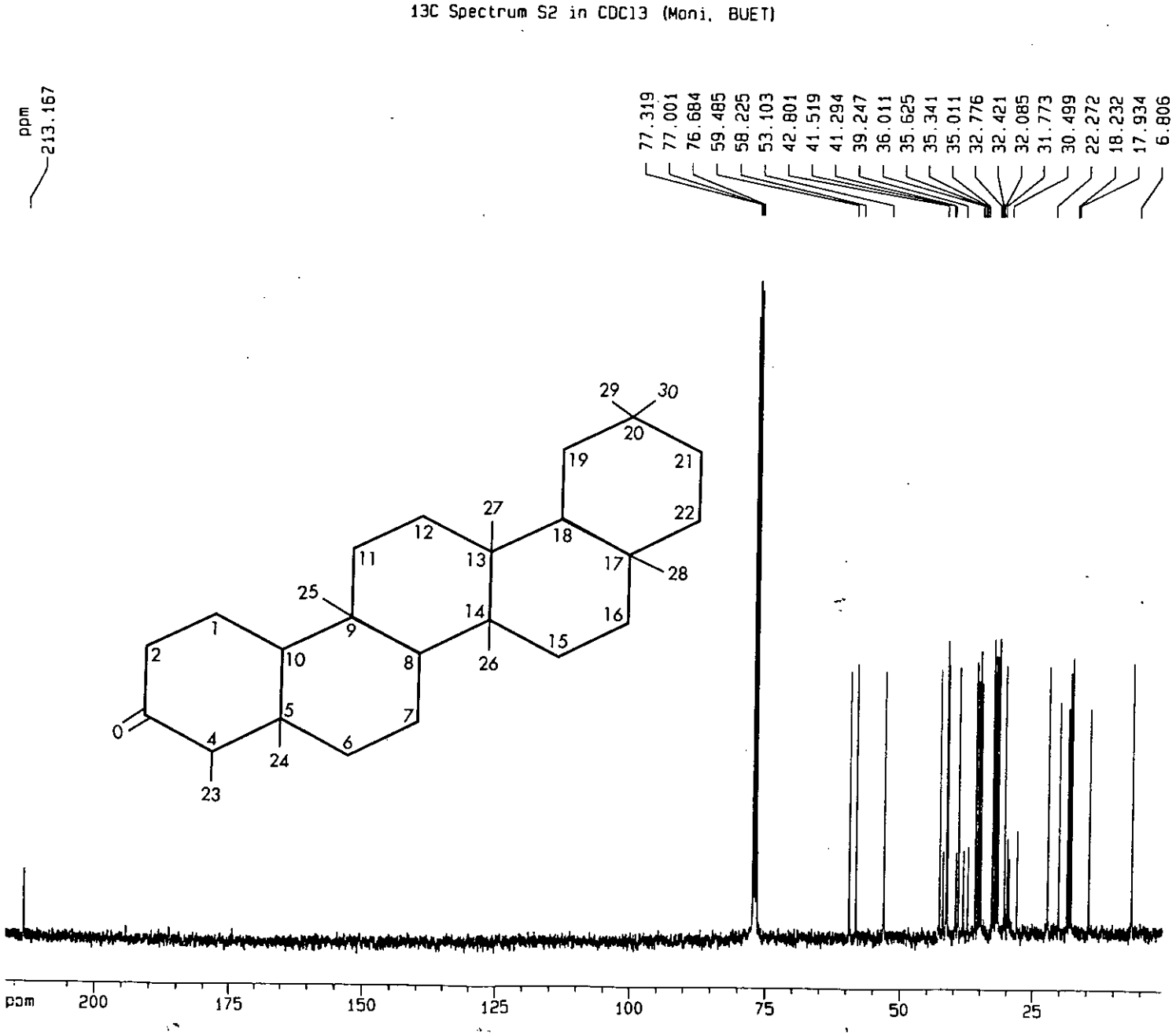
CX	20.00 cm
F1P	10.727 ppm
F1	4292.13 Hz
F2P	-0.086 ppm
F2	-34.41 Hz
PPMCM	0.54063 ppm/cm
HZCM	216.32703 Hz/cm

Fig. : (24) ^1H Spectrum of Sample S_2 in $CDCl_3$



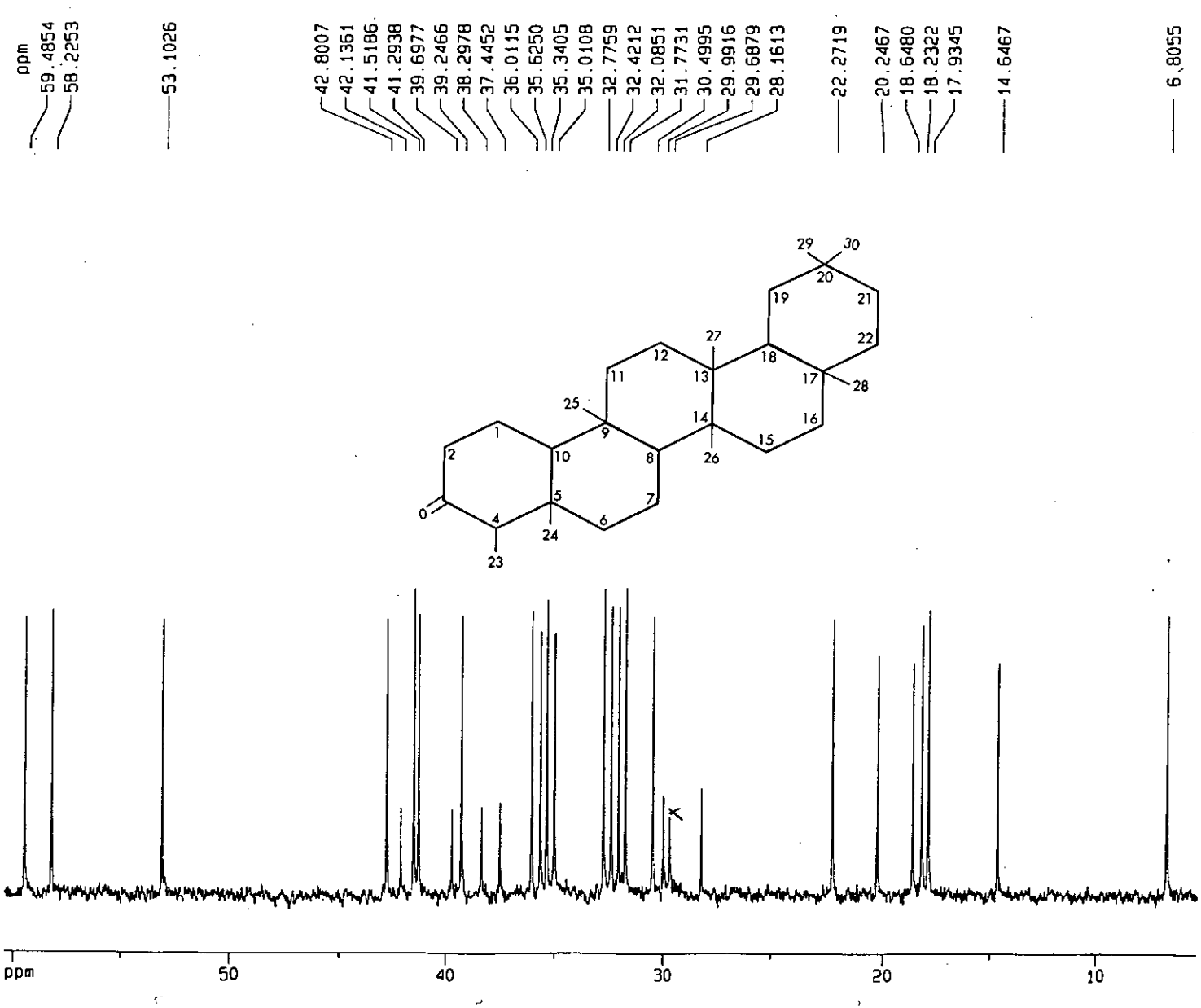

```
Current Data Parameters
NAME                  A120
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_              20010228
Time                 12.03
INSTRUM              dpx400
PROBHD     5 mm Multinuc
PULPROG                 zg
TD                   32768
SOLVENT              CDCl3
NS                      32
DS                       0
SWH               4789.272 Hz
FIDRES            0.146157 Hz
AQ                3.4210291 sec
RG                      64
DW                104.400 usec
DE                  6.00 usec
TE                  310.0 K
D1                1.00000000 sec

============ CHANNEL f1 =============
NUC1                    1H
P1                   8.30 usec
PL1                 -6.00 dB
SFO1           400.1420007 MHz

F2 - Processing parameters
SI                   32768
SF             400.1400049 MHz
WDW                     EM
SSB                      0
LB                   0.30 Hz
GB                       0
PC                    1.40

1D NMR plot parameters
CX                  20.00 cm
F1P                  2.474 ppm
F1                  990.07 Hz
F2P                  0.672 ppm
F2                  268.86 Hz
PPMCM              0.09012 ppm/cm
HZCM              36.06050 Hz/cm
```

Fig. : (25) ^{13}C Spectrum of Sample S_2 in $CDCl_3$




```
Current Data Parameters
NAME                  A120
EXPNO                    2
PROCNO                   1

F2 - Acquisition Parameters
Date_              20010228
Time                 14.10
INSTRUM              dpx400
PROBHD     5 mm Multinuc
PULPROG              zgpg
TD                   32768
SOLVENT              CDCl3
NS                    3306
DS                       0
SWH           22075.055 Hz
FIDRES         0.673677 Hz
AQ            0.7422452 sec
RG                   16384
DW               22.650 usec
DE                6.00 usec
TE                300.0 K
D1            1.50000000 sec
d11           0.03000000 sec
d12           0.00002000 sec

========== CHANNEL f1 ============
NUC1                  13C
P1                 6.60 usec
PL1                -6.00 dB
SF01         100.6263107 MHz

========== CHANNEL f2 ============
CPDPRG2           waltz16
NUC2                  1H
PCPD2             80.00 usec
PL2                -6.00 dB
PL12               16.00 dB
PL13              120.00 dB
SF02         400.1400000 MHz

F2 - Processing parameters
SI                   32768
SF           100.6152845 MHz
WDW                  EM
SSB                   0
LB                 2.50 Hz
GB                    0
PC                 1.40

1D NMR plot parameters
CX                20.00 cm
F1P              216.638 ppm
F1              21797.08 Hz
F2P               0.947 ppm
F2               95.28 Hz
PPMCM          10.78455 ppm/cm
HZCM           1085.09021 Hz/cm
```

Fig. : (26) ^{13}C Spectrum of Sample S_2 in $CDCl_3$



Current Data Parameters

NAME	A120
EXPNO	2
PROCNO	1

F2 - Acquisition Parameters

Date_	20010228
Time	14.10
INSTRUM	dpx400
PROBHD	5 mm Multinuc
PULPROG	zgpg
TD	32768
SOLVENT	CDCl3
NS	3306
DS	0
SWH	22075.055 Hz
FIDRES	0.673677 Hz
AQ	0.7422452 sec
RG	16384
DW	22.650 usec
DE	6.00 usec
TE	300.0 K
D1	1.50000000 sec
d11	0.03000000 sec
d12	0.00002000 sec

========== CHANNEL f1 ==========

NUC1	13C
P1	6.60 usec
PL1	-6.00 dB
SFO1	100.6263107 MHz

========== CHANNEL f2 ==========

CPDPRG2	waltz16
NUC2	1H
PCPD2	80.00 usec
PL2	-6.00 dB
PL12	16.00 dB
PL13	120.00 dB
SFO2	400.1400000 MHz

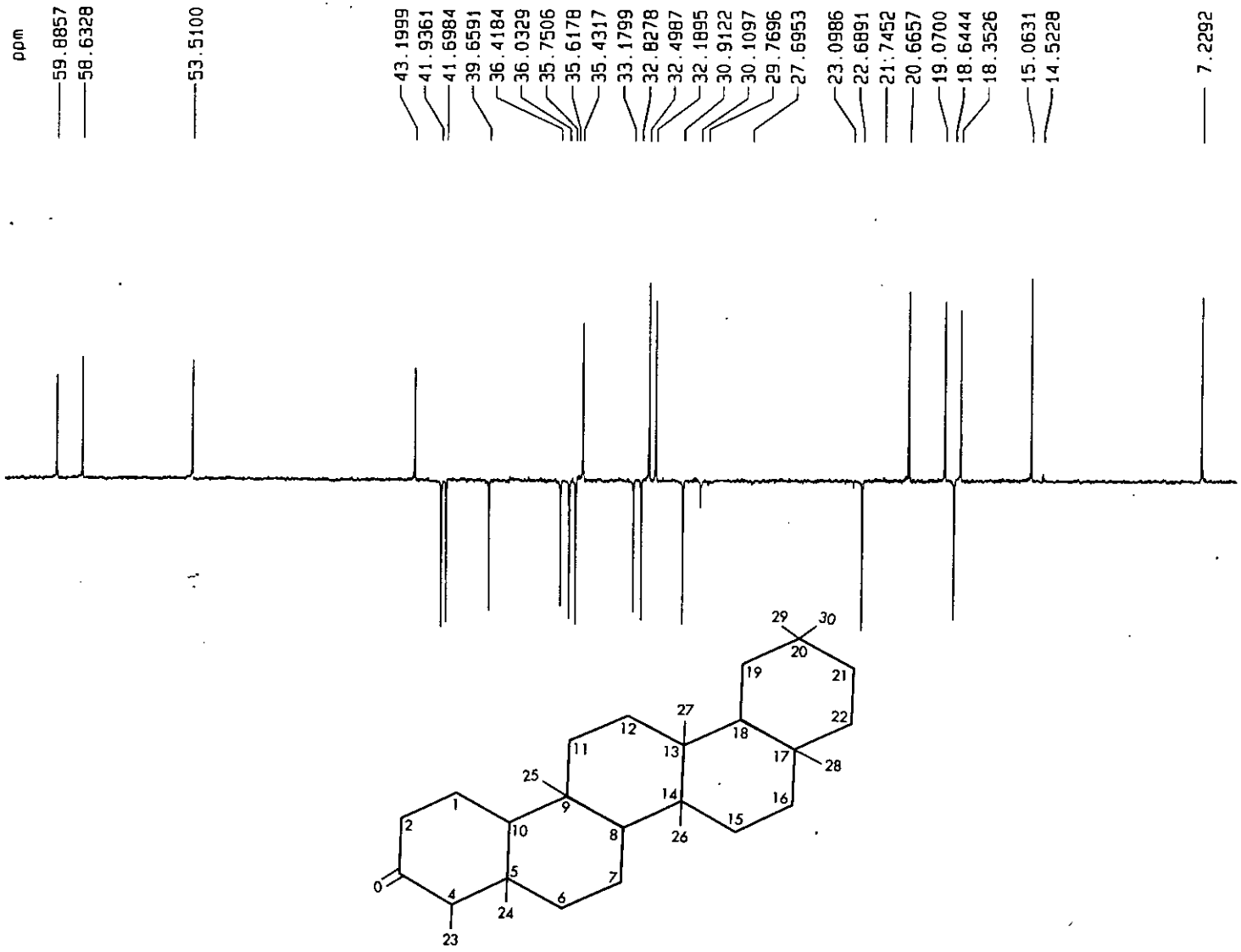
F2 - Processing parameters

SI	32768
SF	100.6152845 MHz
WDW	EM
SSB	0
LB	2.50 Hz
GB	0
PC	1.40

1D NMR plot parameters

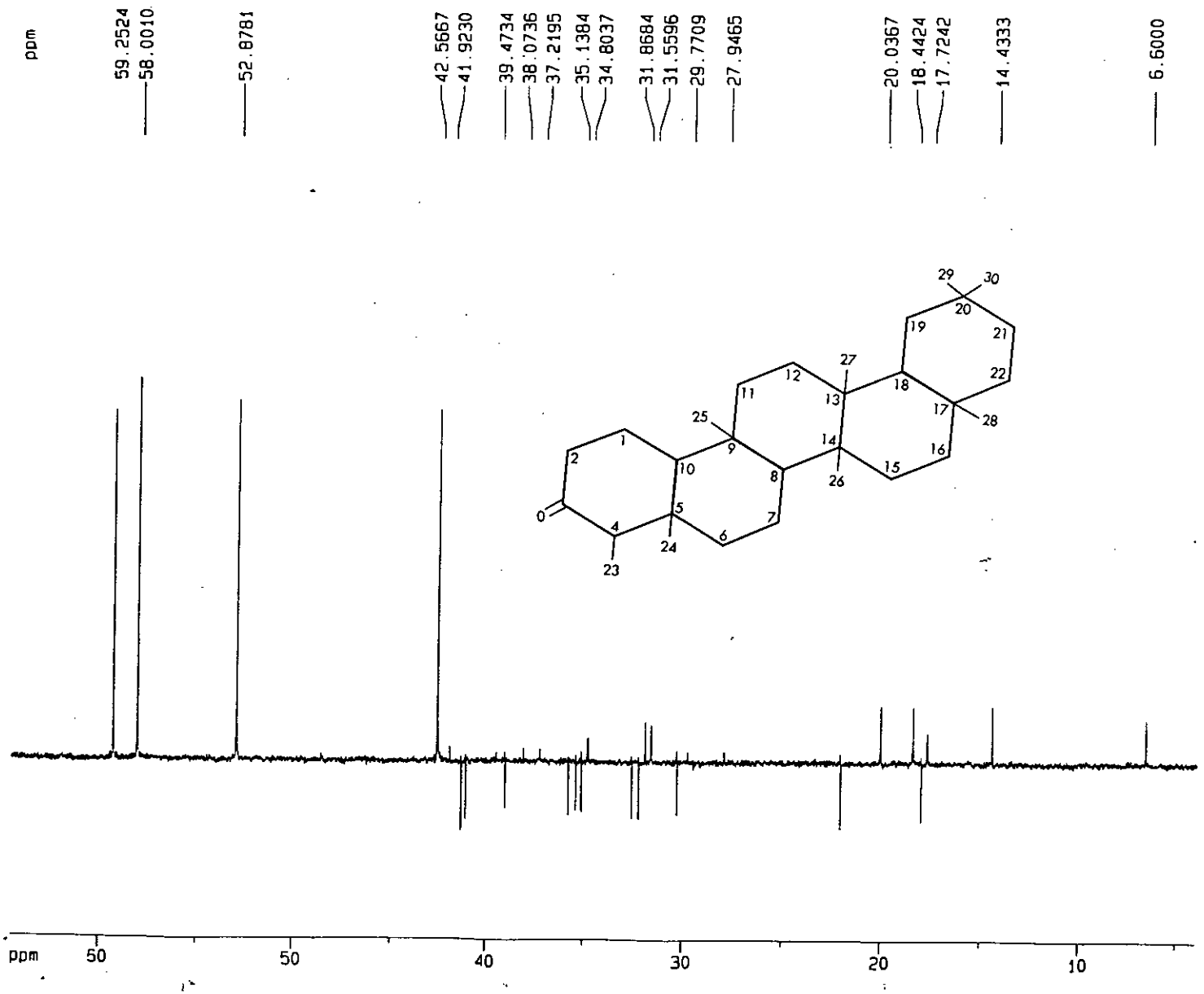
CX	20.00 cm
F1P	60.398 ppm
F1	6077.00 Hz
F2P	5.424 ppm
F2	545.71 Hz
PPMCM	2.74873 ppm/cm
HZCM	276.56445 Hz/cm

Fig. : (27) Dept 135 of Sample S2 in $CDCl_3$




```
Current Data Parameters
NAME                 A120
EXPNO                   3
PROCNO                  1

F2 - Acquisition Parameters
Date_             20010228
Time                 23.30
INSTRUM              dpx400
PROBHD     5 mm Multinuc
PULPROG            dept135
TD                   32768
SOLVENT              CDCl3
NS                    6000
DS                       8
SWH           22075.055 Hz
FIDRES         0.673677 Hz
AQ             0.7422452 sec
RG                   13004
DW                22.650 usec
DE                 6.00 usec
TE                300.0 K
CNST2         145.0000000
D1              4.00000000 sec
d2              0.00344828 sec
d12             0.00002000 sec
DELTA           0.00000764 sec

============ CHANNEL f1 =============
NUC1                 13C
P1                 6.00 usec
p2                12.00 usec
PL1               -6.00 dB
SFO1          100.6263107 MHz

============ CHANNEL f2 =============
CPDPRG2          waltz16
NUC2                 1H
P3                 8.30 usec
p4                16.60 usec
PCPD2             80.00 usec
PL2               -6.00 dB
PL12              16.00 dB
SFO2          400.1420007 MHz

F2 - Processing parameters
SI                   32768
SF            100.6152430 MHz
WDW                  EM
SSB                  0
LB                1.00 Hz
GB                   0
PC                1.40

1D NMR plot parameters
CX                20.00 cm
F1P               62.387 ppm
F1                6277.06 Hz
F2P               5.626 ppm
F2                566.06 Hz
PPMCM          2.83803 ppm/cm
HZCM          285.54956 Hz/cm
```

Fig. : (28) Dept 90 of Sample S2 in $CDCl_3$




```
Current Data Parameters
NAME                 A120
EXPNO                   4
PROCNO                  1

F2 - Acquisition Parameters
Date_             20010301
Time                 7.27
INSTRUM              dpx400
PROBHD     5 mm Multinuc
PULPROG              dept90
TD                   32768
SOLVENT              CDCl3
NS                   6000
DS                      8
SWH              22075.055 Hz
FIDRES           0.673677 Hz
AQ               0.7422452 sec
RG                  14596.5
DW                  22.650 usec
DE                   6.00 usec
TE                  300.0 K
CNST2           145.0000000
D1               4.00000000 sec
d2               0.00344828 sec
d12              0.00002000 sec
DELTA            0.00000764 sec

========== CHANNEL f1 ==========
NUC1                  13C
P1                  6.00 usec
p2                 12.00 usec
PL1                -6.00 dB
SFO1          100.6263107 MHz

========== CHANNEL f2 ==========
CPDPRG2            waltz16
NUC2                  1H
P3                  8.30 usec
p4                 16.60 usec
PCPD2              80.00 usec
PL2                -6.00 dB
PL12               16.00 dB
SFO2          400.1420007 MHz

F2 - Processing parameters
SI                   32768
SF            100.6153064 MHz
WDW                    EM
SSB                    0
LB                  1.00 Hz
GB                     0
PC                  1.40

1D NMR plot parameters
CX                  20.00 cm
F1P                64.589 ppm
F1                 6498.63 Hz
F2P                3.909 ppm
F2                 393.33 Hz
PPMCM             3.03398 ppm/cm
HZCM              305.26492 Hz/cm
```

*** CLASS-5000 *** Report No. = 1 Data : D.D30 01/03/01 15:37:20
Sample : S1
ID : 1ppm
Sample Amount : 1
Dilution Factor : 1
Type : Unknown
Operator : Dr.Mozaffar
Method File Name : MONI.MET

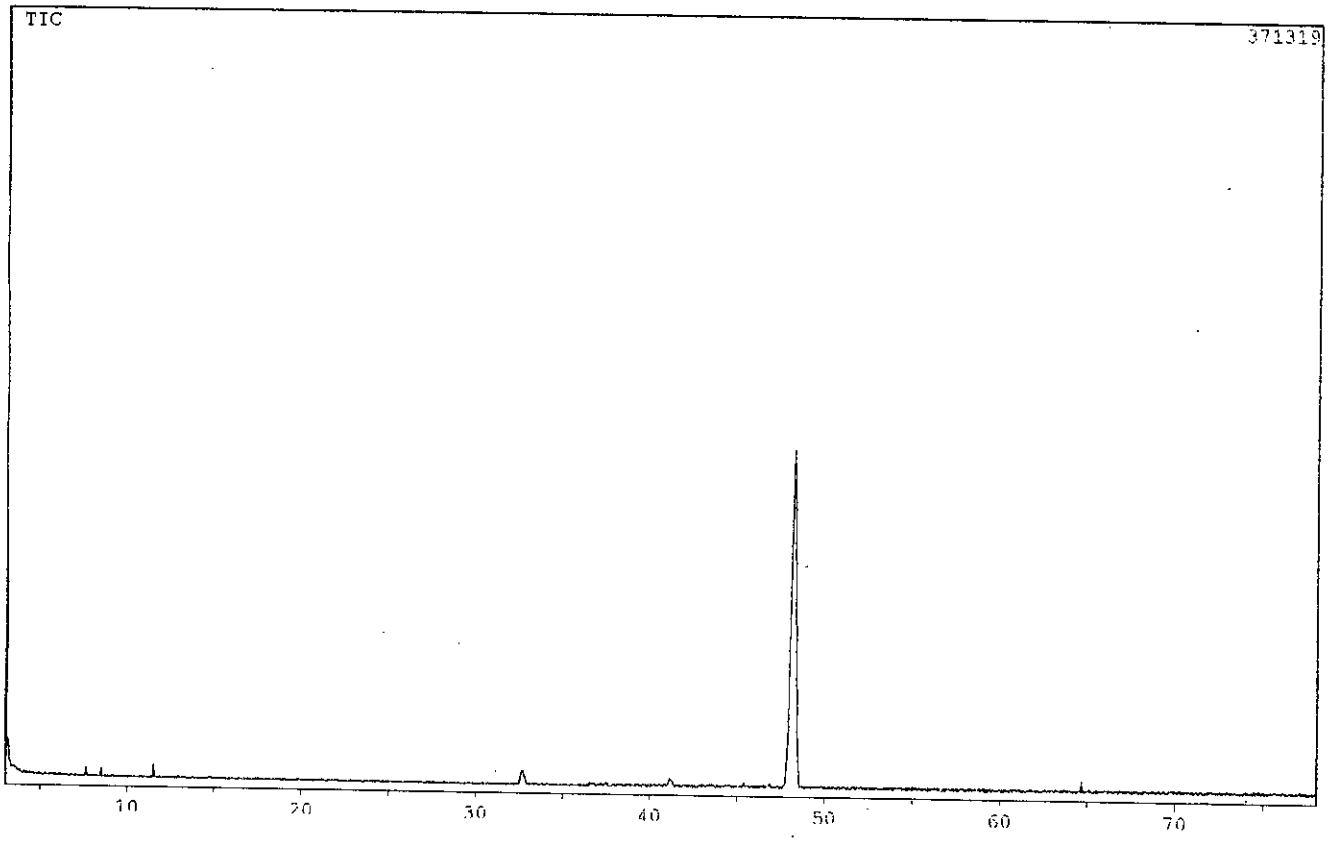
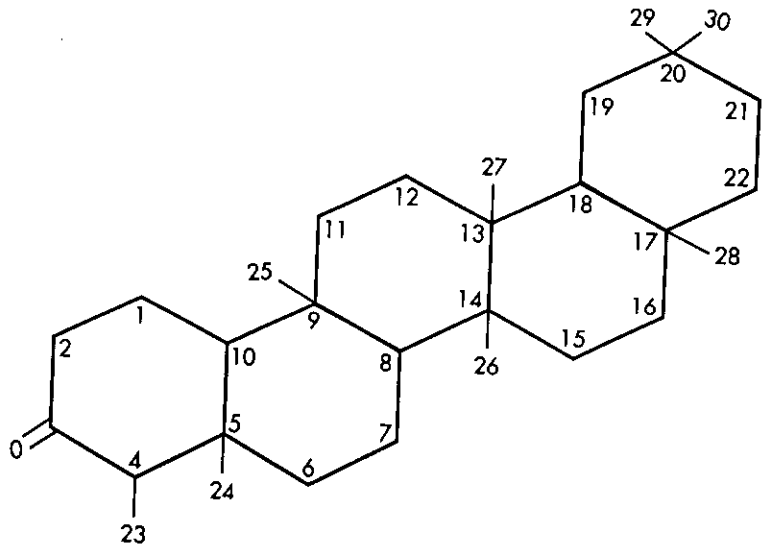


Fig. : (29) GS-Mass Spectrum of Sample S_1

<Unknown Spectrum>
Data : D.D28
Mass Peak # : 133 Ret. Time : 39.858
Scan # : 4424 B.G. Scan # : (4445 - 4456)
Base Peak : 69.15 (39281)



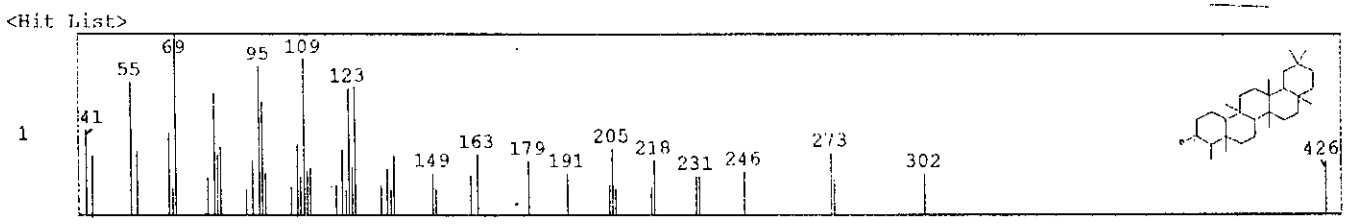


Fig. : (30) GS-Mass Spectrum of Sample S_1

ANALYTICAL RESEARCH DIVISION

*** CLASS-5000 *** Report No. = 1 Data : D.D28 01/02/28 10:39:22
Sample : S2
ID : 1ppm
Sample Amount : 1
Dilution Factor : 1
Type : Unknown
Operator : Dr.Mozaffar
Method File Name : MONI.MET

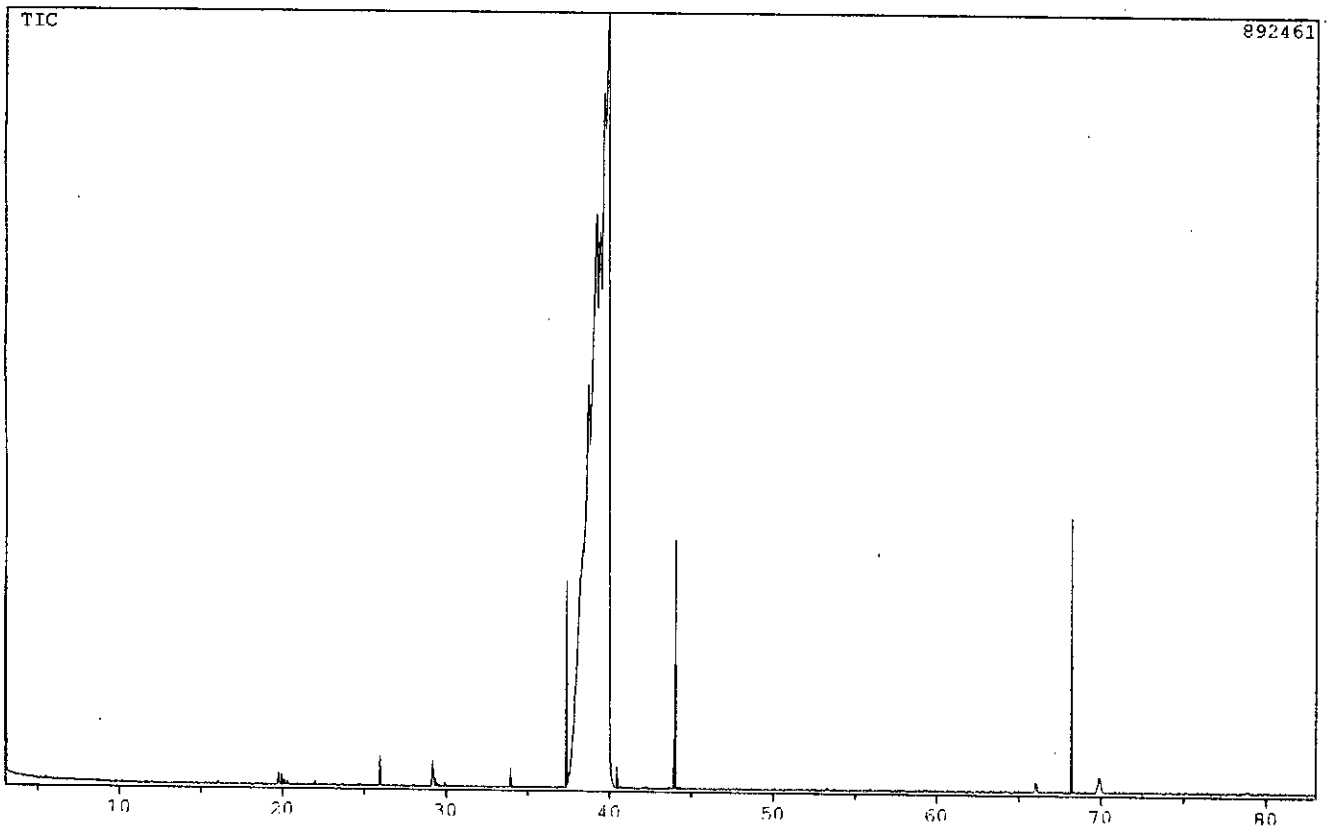
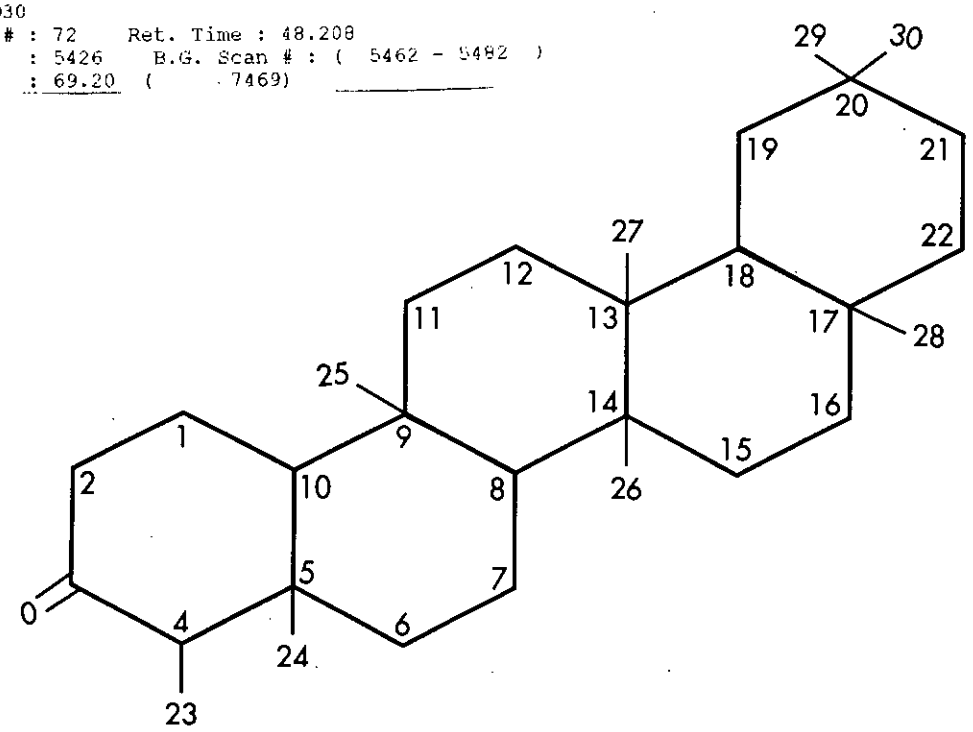


Fig. : (31) GS-Mass Spectrum of Sample S_2

<Unknown Spectrum>
Data : D.D30
Mass Peak # : 72 Ret. Time : 48.208
Scan # : 5426 B.G. Scan # : (5462 - 5482)
Base Peak : 69.20 (7469)



<Hit List>

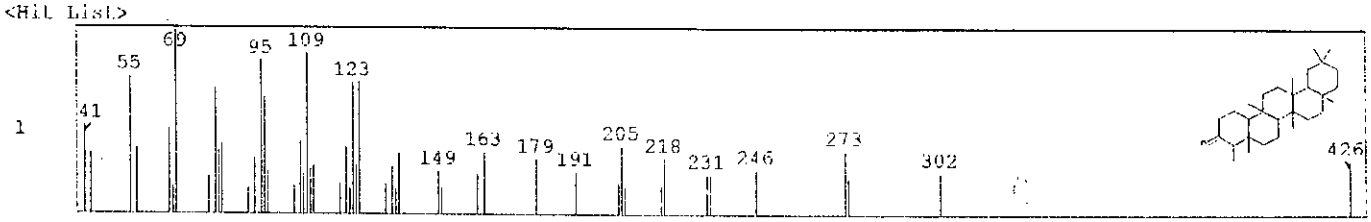


Fig. : (32) GS-Mass Spectrum of Sample S_2

CHAPTER - 3

CHAPTER - 3

3. RUSULTS AND DISCUSSION

3.1. Collection of plant materials :

The leaves and twigs of *V. quadrangularis* were collected from Barisal and Bangladesh University of Engineering & Technology campus, Dhaka in January (2000) with the help of a Taxonomist from the BNH (Bangladesh National Herbarium), Dhaka and identified. The plants were brought to our research laboratory. The samples were sliced in small pices and well dried at room temperature to ready for extraction.

3.2. Isolation of compound (S_1) from Vitis quadrangularis :

The dried sample (503.56 gm) was divided into two parts and ready for extraction. One half (251.78 gm) was kept for methanol (Sec 2.1.11) and another half (251.78 gm) for pet. ether extraction (See 2.1.22). The residue of pet. ether was then extracted with chloroform (Sec 2.1.22). Pet. ether, chloroform and methanol extract was concentrated under reduced pressure and 16.16gm methanollic extracts, 6.0 gm pet. ether extracts and 5.0 gm chloroform extracts were collected.

3.3. Fractionation of methanol extract 'M' by cloumn chromatography :

The methanolic extract (16.16 gm) of the plants *Vitis quadrangularis* was fractionated by silica gel column chromatography using pet. ether and ethylacetate at various ratios followed by methanol as mobile phase (Sec1.1.13). Three fractions were collected on the basis of R_f value (Table - 1)
collection from 35 to 59 fraction giving M_1 was preserved for purification.

3.4. Purification of fraction M_1 :

The fraction M_1 gave a single spot with tailing (Table 1). This fraction was washed repeatedly with pet. ether to remove slight impurities and fatty materials. After washing, white residue was obtained. The residue was collected and named as S_1 (0.92 gm). The compound was characterized by spectroscopic methods and from its physical properties (Sec 2.1.21).

3.5. Characterization of the compound S_1 :

3.5.1. Physical characteristics of S_1 :

The compound S_1 was a white crystalline substance having a melting point of 240-243°C. It was readily soluble in chloroform. It gave a characteristic violet colour of terpenoid with vanillin- sulfuric acid reagent [42].

3.5.2. CHARACTERIZATION OF THE COMPOUND S_1 BY SPECTROSCOPIC METHOD :

3.5.3. IR SPECTROSCOPY

The infrared spectra (Fig.12,13,14) of the compound S_1 run as KBr pellets showed characteristic absorbances at 2915 cm^{-1}, 1725 cm^{-1}, 1450 cm^{-1}, and 1370 cm^{-1}, due to C-H stretching vibration and C=O stretching vibration respectively. The absorbance at 1245 cm^{-1} was due to C-O stretching vibration. The absorbance at 720 cm^{-1} (with in ring or in an open chain) due to methylene group.

3.5.4. ^{1}H- NMR SPECTROSCOPY :

The ^{1}H-NMR spectra (Fig. 16,17,18) of the compound S_1 showed a sharp singlet at δ 0.73 for the methyl protons (3H) of H-24. Another sharp singlet at δ 0.87 for the methyl protns (3H) of H-25.

The compound showed a doublet at δ 0.89 for the methyl protons (3H) of H-23 due to coupling (J=6.5 Hz) with neighbouring proton H-4.

Again, in the ^{1}H-NMR spectra of the compound S_1, five sharp singlets were observed at δ-value 1.01, 1.00, 1.18, 1.05 and 0.96 for the five methyl group at H-26,H-27,H-28, H- 29, andH-30 respectively.

The compound S_1 showed a multiplet at ∂ 1.96 for the proton at H-la. Another multiplet at δ 2.28 was obsersved for the proton of H-2b. A multiplet at δ 2.38 was oberved for the proton of H-2b which is slightly deshielded from the position of H-2a due to influence of neighbouring carbonyl group.

The compound S_1 showed a multiplet at ∂ 1.2-1.8 for the remaining protons (22H).

The ^{1}H-NMR data of the compound S_1 was compared with that of friedelan-3-one [43] and found to be in good agreement with the data published in the literature [43,44,45].

3.5.5. ^{13}C-NMR SPECTROSCOPY :

The ^{13}C-NMR spectra (fig 19,20,21,22) of the compound S_1 showed thirty signals indicating terpenoid nature of the compound.
The ^{13}C- NMR spectrum (Fig17,18,19) of the compound was expanded and DEPT technique was employed to distinguish among the nature of thirty carbons. By using 135° and 90° DEPT experiments, distinction among methylene and methine carbons was found out. It was found taht the signals at δ 6.80, 14.64, 17.93, 20.24, 18.64, 32.08, 35.01 and 31.77 were due to ten methylene carbons of C-23, C-24, C-25, C-26, C-27, C-28, C-29 and C-30 respectively. The signals at ∂ 22.27, 41.51, 41.29, 18.23, 35.62, 30.49, 32.42 36.01, 35.34, 32.77 and 39.24 were observed due to eleven methylene cartons at C-1, C-2, C-6, C-7, C-11, C-12, C-15, C-16, C-19, C-21 and C-22 respectively. The signals at ∂ 58.22, 53.10, 59.48 and 42.80, were due to four methine carbons at C-4, C-8, C-10 and C-18 respectively . The signals at ∂ 42.14, 37.44, 39.69, 38.29, 29.99 and 28.16 were due to six quarternary carbons at C-5, C-9, C-13, C-14, C-17 and C-20 respectively.

Table -4

^{1}H-NMR SPECTRAL DATA OF THE COMPOUND S_1 (Chemical Shift in ppm).

Position	δ Values in ppm	Splitting Pattern	Integration	Assignment	Reported data
H-24	0.73	Singlet	3H	Methyl proton	0.71 (S, 3H)
H-25	0.87	Singlet	3H	CH_3- proton	0.85 (S,)
H-23	0.89	Doublet	3H	CH_3- proton	0.87 (S, 3H)
H-26	1.01	Singlet	3H	CH_3- proton	1.00 (S)
H-27	1.00	Singlet	3H	CH_3- proton	1.00 (S)
H-28	1.18	Singlet	3H	CH_3- proton	1.17 (S)
H-29	1.05	Singlet	3H	CH_3- proton	1.05 (S)
H-30	0.96	Singlet	3H	CH_3- proton	0.95 (S)
H-1a	1.96	Multiplet	1H	Methylene proton	1.96 (M)
H-2a	2.28	Multiplet	1H	Methylene proton	2.28 (M)
H-2b	2.38	Multiplet	2H	Methylene proton	2.38 (M)

From 1.2 - 1.8 multiplet include rest of the protons (22H).

Table -5

^{13}C-NMR SPECTRAL DATA OF SAMPLE S_1 :

No. carbon	Types of Carbon	Chemical Shift in δ PPM	Reported data (Friedelin) ^{13}C δ ppm
1	$-CH_2-$	22.27	22.3
2	$-CH_2-$	41.51	41.5
3	>CO	213.16	213.2
4	$-CH_2-$	58.22	58.2
5	>C<	42.14	42.1
6	$-CH_2-$	42.29	41.3
7	$-CH_2-$	18.23	18.2
8	>CH–	53.10	53.1
9	>C<	37.44	37.4
10	>CH–	59.48	59.4
11	$-CH_2-$	35.62	35.6
12	$-CH_2-$	30.49	30.5
13	>C<	39.69	39.7
14	>C<	38.29	38.3
15	$-CH_2-$	32.42	32.4
16	$-CH_2-$	36.01	36.0
17	>C<	29.99	30.0
18	>CH–	42.80	42.8
19	$-CH_2-$	35.34	33.3
20	>C<	28.16	28.1
21	$-CH_2-$	32.77	32.7
22	$-CH_2-$	39.24	39.2
23	$-CH_3$	6.802	6.8
24	$-CH_3$	14.64	14.6
25	$-CH_3$	17.93	17.9
26	$-CH_3$	20.24	20.2
27	$-CH_3$	18.64	18.6
28	$-CH_3$	32.07	32.1
29	$-CH_3$	35.00	35.0
30	$-CH_3$	31.77	31.8

95414

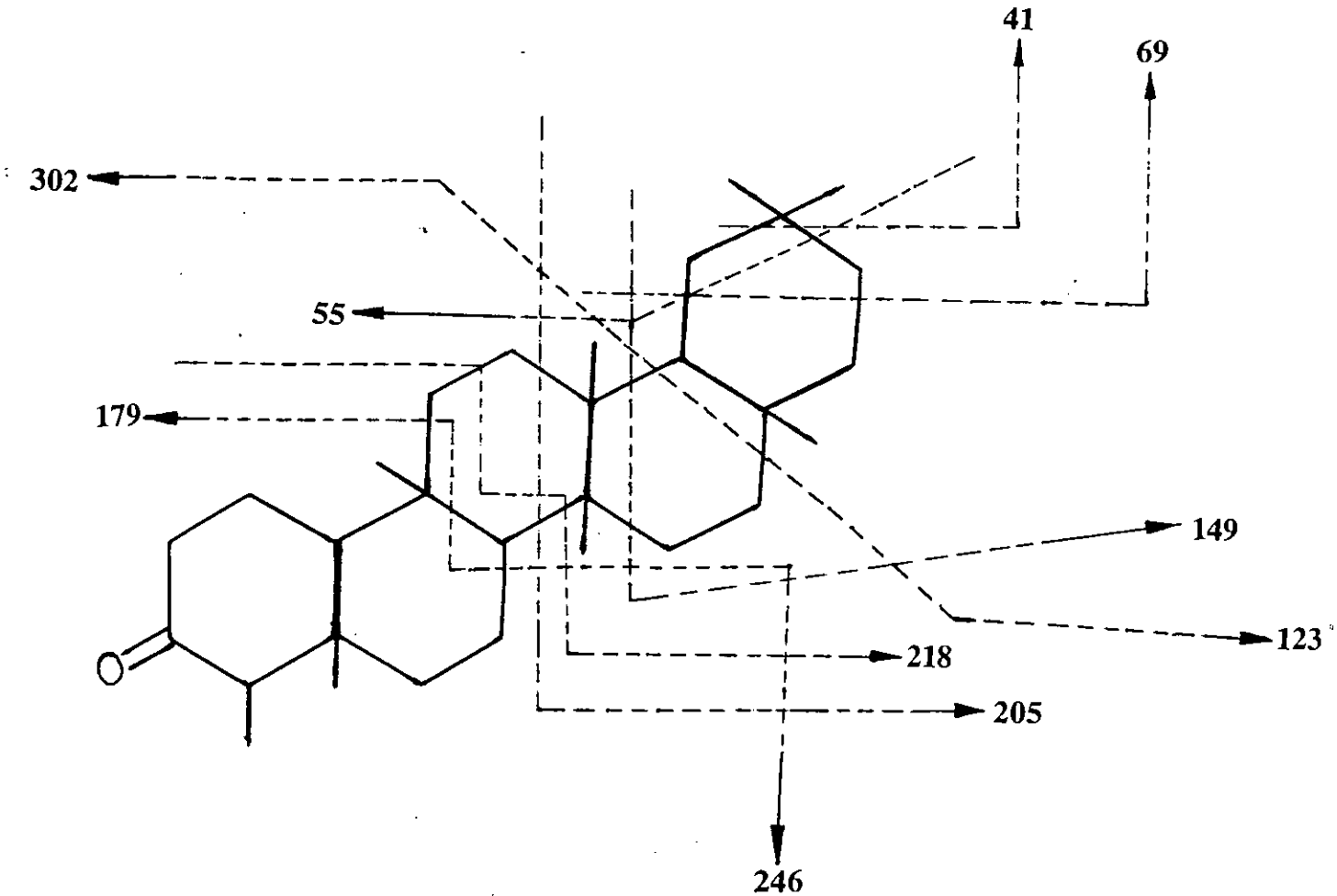


Fig. : (33) GC-Mass Spectrum Fragmentation of Sample S_1

The signals at ∂ 6.802, 14.64, 17.93, 20.24, 18.64, 32.07, 35.00, 31.77 were due to eight methyl carbons of C-23, C-24, C-25, C-26, C-27, C-28, C-29 and C-30 respectively.

One characteristic signals at ∂ 213.16 were observed due to corbonyl carbon of C-3.
The ^{13}C- NMR data of compound S_1 were compared with that of friedelin compound (Table 5) published in the litereture. It was found that ^{13}C-NMR data of compound S_1 were very much in agreement with the ^{13}C-NMR data of friedelin.[43]

3.5.6. GC - MASS SPECTRAL ANALYSIS OF SAMPLE S_1:

GC-MS SPECTRAL DATA : 426 $M^{\pm}$

302, $[M-C_9H_{15}]^+$, 246 $[M-C_{12}H_{19}O]^+$, 205 $[M-C_{15}H_{25}]^+$, 218 $[M-C_{16}H_{26}]^+$, 149 $[M-C_{11}H_{17}]^+$, 179 $[M-C_{12}H_{19}O]^+$, 123 $[M-C_9H_{15}]^+$, 95 $[M-C_7H_{11}]^+$, Base peak 69 $[M-C_5H_9]$+, 55 $[M-C_4H_7]^+$, 41 $[M-C_3H_5]^+$.

The mass spectra (Fig. 29,30) of S_1 had a molecular ion peak, M^+ at m/z 426 indicating 426 as the molecular mass of the compound. This molecular ion peak also corrsponded to a molecular formula $C_{30}H_{50}O$. The base peak was at m/z 69 and other more intense peaks were at m/z 302, 246, 205, 218, 149, 179, 123, 95, 55 and 41.

By comparing IR, ^1H-NMR, ^{13}C-NMR and GC-MS data, the compound S_1 was characterized as Friedelan-3-one and the stracture of the compound was established[43] as following (Fig 34).

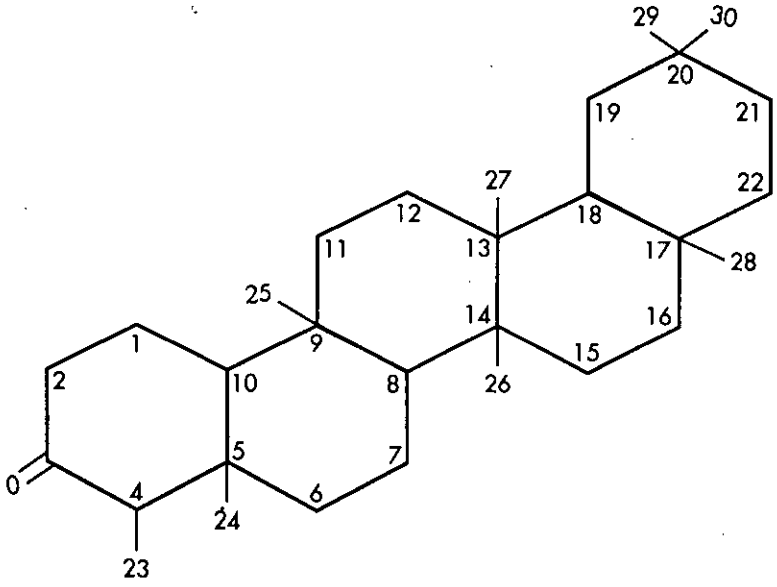


Fig. (34) : Friedelin D:A-Friedooleanan-3-one

3.6. FRACTIONATION OF PET. ETHER EXTRACT (P) BY COLUMN CHROMATOGRAPHY :

The pet. ether extract (6.0 gm) of crude brownish coloured gummy mass was fractionated by silica-gel column chromatography using pet. ether methanol and ethylacetate as mobile phase (Sec2.1.24). Two fractions were collected on the basis of R_f value (Table 2). Collection no. 20 to 55 fracton P_1 was preserved for purification.

3.7. PURIFICATION OF FRACTION P_1 :

The fraction P_1 gave a single spot with tailing (Table 2). This fraction was washed repeatedly with pet. ether to remove slight impurities and fatty materials. After washing white residue was obtained. The residue was dried, collected and named as S_2 (0.78 gm). The compound was characterized by spectroscopic and physical properties.

3.8. CHARACTERIZATION OF THE COMPOUND S_2 :

3.8.1. PHYSICAL CHARACTERISTICS OF S_2 :

The compound S_2 was a white crystalline substance having a melting point of 240-242°C. It was readily soluble in chloroform. It gave characteristic violet colour of terpenoid with vanillin- slfuric acid reagent[42].

3.8.2. CHARACTERIZATION OF THE COMPOUND S_2 BY SPECTROSCOPIC METHOD :

3.8.3. IR SPECTROSCOPY

The infrared spectra (Fig 15) of the compound S_2 run as KBr pellets showed characteristic absorbances at 2915 cm^{-1}, 1725 cm^{-1} and at 1350 cm^{-1} due to C-H stretching vibration and C=O stretching vibration respectively. The absorbance at 1245 cm^{-1} was due to C-O stretching vibration. The absorbance at 782 cm^{-1} (within a ring or in an open chain) due to methylene group.

3.8.4. ^{1}H NMR SPECTROSCOPY :

The ^{1}H-NMR spectra (Fig. 16,17,18) of the compound S_2 showed a sharp singlet at δ 0.73 for the methyl protons (3H) of H-24. Another sharp singlet at δ 0.87 for the methyl protns (3H) of H-25.

The compound showed a doublet at δ 0.89 for the methyl protons (3H) of H-23 due to coupling (J=6.5 Hz) with neighbouring proton H-4.

Again, in the ^{1}H-NMR spectra of the compound S_2, five sharp singlets were observed at δ-value 1.01, 1.00, 1.18, 1.05 and 0.96 for the five methyl group at H-26,H-27,H-28, H- 29, andH-30 respectively.

The compound S_2 showed a multiplet at ∂ 1.96 for the proton at H-la. Another multiplet at δ 2.28 was obsersved for the proton of H-2b. A multiplet at δ 2.38 was oberved for the proton of H-2b which is slightly deshielded from the position of H-2a due to influence of neighbouring carbonyl group.

The compound S_2 showed a multiplet at ∂ 1.2-1.8 for the remaining protons (22H).

The ^{1}H-NMR data of the compound S_2 was compared with that of friedelan-3-one [43] and found to be in good agreement with the data published in the literature [43,44,45].

3.8.5. 13C-NMR SPECTROS COPY :

The ^{13}C-NMR Spectra (fig 23,24,25) of the compound S_2 showed thirty signals an indicative of terpenoid nature of the compound. The ^{13}C- NMR spectrum (Fig 26,27,28) of the compound was expanded and DEPT technique was employed to distinguish the nature of

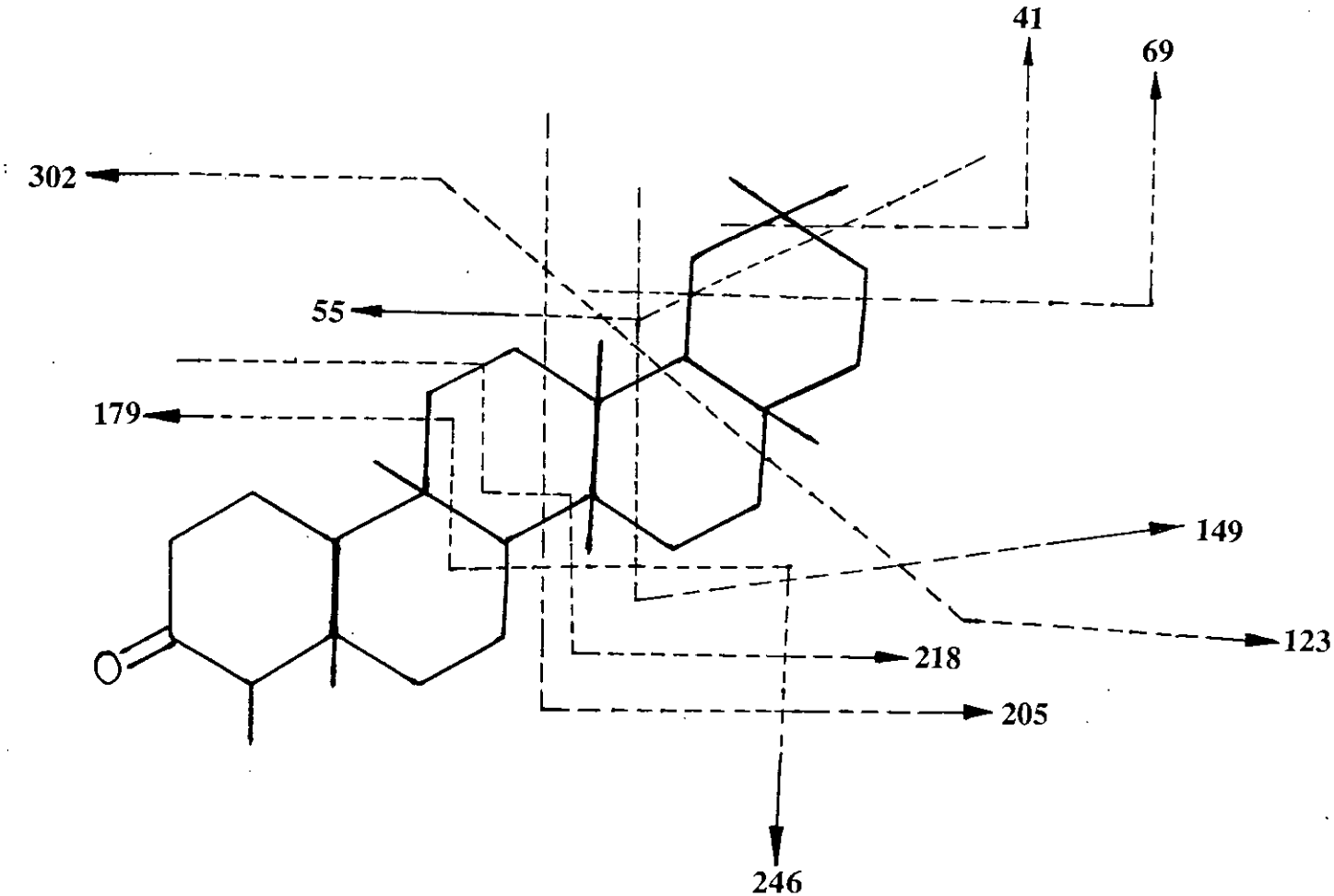


Fig. : (35) GC-Mass Spectrum Fragmentation of Sample S_2

thirty carbons. By using 135° and 90° DEPT experiments distinction among methylene and methine carbons was found out.

It was found taht the signals at δ 6.80, 14.64, 17.93, 20.24, 18.64, 32.08, 35.01 and 31.77 were due to ten methylene carbons of C-23, C-24, C-25, C-26, C-27, C-28, C-29 and C-30 respectively. The signals at δ 22.27, 41.51, 41.29, 18.23, 35.62, 30.49, 32.42 36.01, 35.34, 32.77 and 39.24 were observed due to eleven methylene casbons of C-1, C-2, C-6, C-7, C-11, C-12, C-15, C-16, C-19, C-21 and C-22 respectively. The signals at δ 58.22, 53.10, 59.48 and 42.80, were due to four methine carbons of C-4 C-8, C-10 and C-18 respectively . The signals at δ 42.14, 37.44, 39.69, 38.29, 29.99 and 28.16 were due to six quaternary casbons of C-5, C-9, C-13, C-14, C-17 and C-20 respectively.

The signals at δ 6.802, 14.64, 17.93, 20.24, 18.64, 32.07, 35.00, 31.77 were due to eight methyl carbons of C-23, C24, C-25, C-26, C-27, C-28, C-29 and C-30 respectively.

One characteristic signal at ∂ 213.16 was obtained due to carbonyl carbon of C-3. The ^{13}C-NMR data of compound S_2 was compared with that of friedelin Compound (Table 5) published in the literature. It was found that ^{13}C-NMR data of compound S_2 were very much in agreement with the ^{13}C-NMR data of friedelin.

3.5.6. GC - MASS SPECTRAL ANALYSIS :

GC-MS SPECTRAL DATA : 426 $M^{\pm}$

302, $[M-C_9H_{15}]^+$, 246 $[M-C_{12}H_{19}O]^+$, 205 $[M-C_{15}H_{25}]^+$, 218 $[M-C_{16}H_{26}]^+$, 149 $[M-C_{11}H_{17}]^+$, 179 $[M-C_{12}H_{19}O]^+$, 123 $[M-C_9H_{15}]^+$, 95 $[M-C_7H_{11}]^+$, Base peak 69 $[M-C_5H_9]$+, 55 $[M-C_4H_7]^+$, 41 $[M-C_3H_5]^+$.

The mass spectra (Fig. 29,30) of S_2 had a molecular ion peak, M^+ at m/z 426 indicating 426 as the molecular mass of the compound. This molecular ion peak also corrsponded to a molecular formula of $C_{30}H_{50}O$. The base peak was at m/z 69 and other more intense peaks were at m/z 302, 242, 205, 218, 149, 179, 123, 95, 55 and 41.

By comparing IR, 1H-NMR, ^{13}C-NMR and GC-MS data, the compound S_2 was characterized as Friedelan-3-one and the structure of the compound was established[43] as following (Fig. 36).

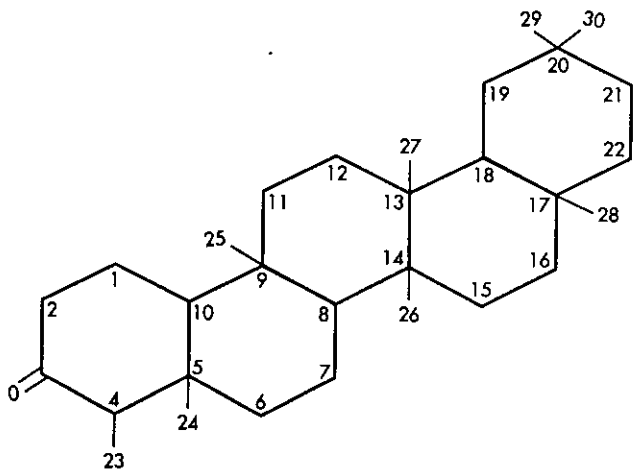


Fig. (36) : Friedelin D:A-Friedooleanan-3-one

CHAPTER - 4

CHAPTER - 4

4.1 A BRIEF REVIEW OF FRIEDELIN AND ASSOCIATED TRITERPENOIDS :

literature is reviewed for the natural occurrence of the pentacyclic triterpenes, friedelin and the epimeni 3 - friedelanols. A summary of the literature relating to the structure elucidation, physical properties is presented. Compounds possessing the same carbon sketeton (D:A-friedelooleanane) and occuring with at least one of the above friedelane derivatives are tabulated.

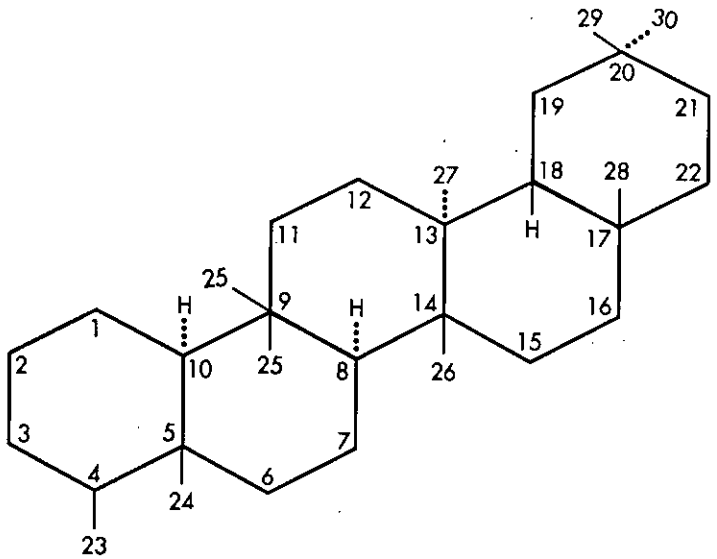


Fig. (37) : Skeleton and numbering of the friedelanes, (D:A-Friedooleanane.)

The scientific literature on friedelin, the 3-keto-derivative of the hydrocarbon, friedelane (Fig 35), and related plant sources are included in this review.

A survey of the occurrence and strucetures of triterpenoids for the period 1963-70 was presented by Kulshreshtha et al[46].

The only comprehensive review with friedelin as the focus is provided by Sainbury [47]. Although his article lists only 73 sources of friedelin, it also documents the associated occurrence of the epemeric B-friededanols and other triterpenes.

Friedel[48], according to karrer [49], first isolated friedelin ; however, a much earlier reference is provided by Elservier [50] to chevreul[51] who obtained a substance from cork whch he called 'Cerine'. In 1899 Istraiaol Ostrogovich[52] showed that 'Cerine' was composed of two substances, one they named friedelin in honor of their friend Friedel and Cerin.

By 1935, Draka and Jacobsen[53] had repeated the isolation of friedelin and cerin, determined their molecular weights and empirical formulae and proven a common skeleton.

Drake, working with Shrader[54] and Haskins[55] presented evidence that friedelin contained a ketone and that cerin was a hydroxyketone. The ensuing years saw numerous reports on studies of friedelin, with chemists preparing derivatives attempting to trace biosynthetic intermediates and trying to synthesize fridelin.

Efforts to elucidate the structure and conformation were based initially on the preparation of derivatives [56-58]. Most modern physical methods were yet to be developed, but Drake and Wolfe[59] investigated the surface films-formed by friedelin and cerin, and Lander and Svirbely[60] studied the structure of friedelin using the dipole moments of cerin, friedelin and various isomers. The latter paper contains an early reference to friedelan -3∞-ol.

In 1944 and 1949 Ruzicka and co-workers[61,62] published the results of their attempts to derive the structure of fridelin by chemical means. However it was not until 1995 that two groups (Dutler, Jeger and Ruzicka[63] and corey any Ursprung[64,65] working independently arrived at the accepted structure as a result of dehydrogenation studies and the isomerization of friedel-3-one to olean-13(18)-ene. The structure was confirmed independently by Brownlie and co-workers[66-68] and Ourisson and co-workers[69]. Further studies of the friedenin-oleanene rearrangement continued with attempts to isolate the intermediate compounds[70-74].

After the structure of friedelin was established studies were done, notably by Djerassi and co-workers[75,76] to determine the configuration using optical rotary dispersion. Acrystallographic study of friedelin was carried out by Rogers and Thomas[77] who reported that the cell contained four molecules the formula indicated was C_{30} H_{50} O and the crystals were orthorhombic. Further studies concluded that there were two possible conformations, the quasi-all-chair and the chair : chair : chair : boat : boat[78]. More recently, the crystal structure and conformation of friedelan-3ß-o1 have been reported and confirmed the latter conformation[79].

Isolation procedures have been refined with the development of chromatography and its application to natural products. Stevenson and kane.[80,81] reported a convenient isolation proedure to-obtaining friedelin and related compounds from corkboard by products.

Further details on column chromatography in the isolation and purification of triterpenes, including friedelin and related compounds, have been reported by Cambie and Parnell [82] and Sengupta and Mukherjee[83].

Several publications deal with TLC of pentacyclic triterpenes. Information regarding solvent systems, detection reagents and R_f values is available[84-86]. Increased efficiency in separating compounds of a given class is obtained on the plates impregnated with silver nitrate as described by Fisher and Hertel.[87] and by Barua et al[88].

Spectroscopic methods have been applied to the study of triterpenes. Ourisson and Takahashi[89] published the IR spectrum of friedelin and Cole et al.[90] reported the spectrum of friedelan-3∞-ol. solvent etfect of IR spectroscopy were reported by Oganesyan et al.[91] and Brooks et al[92].

The development of NMR Spectroscopy and mass spectrometry (MS) resulted in a series of papers reporting, first, the spectra obtained with known compounds and then, the attempts to correlate this knowledge with the spectra of compounds of undetermined structures. Djerassi any co-workers[93-95] were responsible for much of the general application of MS to triperpenes, including friedelin,while courtney et a [96,97], Hunech and Tummler[99], and Hirota et-al[98] considered the problem of friedelin-related derivatives.

Shamma etal[100] published NMR spectra of a series of triterpenoid derivatives with correlations between spectra and structure as did Djerassi[94]. The value of NMR in structure elucidation was increased with the application of solvents induced shifts[101] and the development of shift reagents[102,103], the use of ^{13}C NMR[104-106] and the application of homonuclear INDOR spectroscopy[107,108].

The utility of combining NMR spectroscopy and MS in the determination of friedelin related compounds is well demonstrated by two series of papers. Kikuchi and co-workers[109] isolated friedelin type components from Pachysandras terminalis and formulated the structures based on the spectroscopic evidence of the compounds and their derivatives. The other series by Courting and co-workers, dealt with a number of carbonyl, hydroxycarbonyl and polyhydroxy friedelanes isolated from siphonodon australe[71,72].

A series of papers by Stevenson and his co-workers[110] explored the reactions of friedelane with bromine and with n-bromosuccinimide. Takyuki et al.[111] and Shoppe and Johnson[112] provided interesting comments on yields in these preparations. Stevenson and co-workers[113] went to investigate the effects of ultraviolet light on reactions involving friedelin and its derivatives.

In addition, other workers employed a wide variety of techniques and reactivity of friedelin and related compounds. Among these were the reduction of cerin oxime[114], the preparation of epimeric 3-amino friede lanes the acid catalysed rearrangement of the 3-friedelene into 13(18)-oleane[74], conformation studies of oxo-triterpenes using circular dichroism[116], and survey of the possible rearrangements leading to friedelin-type compounds.

The biosynthesis of triterpenes has been reviewed by Res and Good on[117] and discussed by Sengupta[118].

The chemical synthesis of friedelin starts with a more available triterpene such as amyrim[119] or with readily available tetracyclic tricterpene[120], or attacks the problem through the stereo-specific synthesis of intermediates[121]. A total synthesis of friedelin was reported in 31 steps and 0.3%, yield[122].

USES OF FRIEDELIN :

The uses of friedelin or friedelin type compounds has been considered for treatment of cancer of the bladder[123], convulsion, inflammation[124], topical ulcers, rheumatic inflammation, fever and dysentry[125] ; Friedelin has also been found to have an "antifeedant" activity upon. some insects[126].

OCCURRENCE OF FRIEDELIN :

The natural occurrence of friedelin and the 3-friedelanols is extensive. Friedelin has been reported in many of the higher plants and in algae[127,128], lichens[129,130], mosses, peat[42,43,46,133,134] coal [135-140] and mineral wax(135). It has also been reported in swine[136] and epifriedelinol has been found in microorganisms[137]. Finally a word of caution, the finding of friedelin in a sdudy of polyhydroxylated neutral aglucons[138] was attributed to the cork stoppers used in the extraction :

Table : 6

4.2 The sources used for occurence of friedelin[145]

Name of Plant	Part used
Abroma augusta	bark, roots
Acer amoentum	leaves
Acokanthera spectabilis	various parts
Actinodaphne augustifolia	leaves
Aesculus hippocastanum	seed oil
Ageratum conyzoides	plant material
Ageratum houstonianum	plant material
Alangium lamarckii	leaves
Albizzia lebbeck	bark
Alectoria ochroleuca	whole plant
Alectryon excelsum	bark
Aleurites montana	bark
Antidesma bunius	leaves, stems
Amirrhoea chinensis	leaves
Aporoosa chinensis	leaves, stems
Argyreia speciosa	leaves
Argyreia populifolia	fruit
Arundinaria chino	rhizomes
Arudinaieae	whole plant
Arundinella hirta	herb
Arundo donax	leaves
Asclepias tuberosa	leaves
Aster baccharoides	leaves
Aster baccharoides	stems
Aster scaber	roots
Aster tataricus	roots
Atalantia monophylla	leaves
Athrixia pinilolia	roots
Arieennia offcinallis	aerial parts
Baccaurea sapida	bark
Balanops anstraliana	whole plant
Bischofia jacanica	whole plant
Bischofia trifoliata	bark
Bridelia micrantha	bark
Brideli monica	leaves, stems
Bridelia stpularis	bark
Bromus rigidus	culms, blades

Table : 6 Continued

Name of Plant	Part used
Brosimum (Takini bark)	bark
Byrsoenima rerbascifolia	bark
Cacalta bulbifera	whole plant
Calophyllum apetalum	bark
Calophyllum brasiliense	not stated++
Calophyllum cordato-oblongum	bark, wood
Calophyllum cuneifolium	bark
Calophyllum inophyllum	bark
Calophyllum inophyllum	leaves
Calophyllum chwaiteii	bark
Calophyllum tomentoswn	bark
Cannabis satica	root
Canscora decussata	aerial parts
Canscora decussata	roots
Caraipa densifiord	wood
Carapa oborata	bark, leaves
Caryocar brasiliense	leaves
Castanopus concinna	leaves, stems
Castanopus cuspidata	leaves
Castanopus cuspidata	stems
Castanopus cuspidata var. sieboldii	bark
Castanopis eyrei	leaves, stems
Castanopis fabri	leaves
Castanopis fabri	stems
Castanopis fissa	leaves, stems
Castanopis hickelii	leaves, stems
Castanopis lamonltii	leaves
Castanopis lamontii	stems
Caratopetalum apetaluum	bark
Cetruria crispa	whole plant
Cetruria cuculatta	whole plant
Cetruria delisei	whole plant
Cetruria islandica	whole plant
Cetruria nicalis	whole plant
Choisya ternata	leaves
Citrus aurantium var. sinensis	oil from peel
Citrus paradisi	oil from peel
Cladonia alpestris	whole plant
Claoxylon polot	leaves
Clerodendron trichotomum	bark

Table : 6 Continued

Name of Plant	Part used
Clusia rosea	plant material
Coix lacryma-jobi	herb
Colletia hystrix	not stated++
Colletia spinosa	not stated++
Conocephalum conicum	not stated++
Cuphea balsamona	not stated++
\Cynanchum rincetoxicum	not stated++
Cynodon dactylon	herb
Dalbergia colubilis	wood chips
Dichrosatchys cinerea	bark, roots
Diospyros baxifolia	leaves, stems
Diospyros kaki	flowers
Elaeocarpus hookerianus	wood
Elephantopus scaber	plant material
Erica arbored	not stated++
Erythrospermum ceylanicum	bark
Eugenia fructicosa	bark
Eugenia jambolana	stem bark
Eugenia wallichu	bark
Euonymus alata	leaves
Euonymus faponica	leaves
Euonymus radicans	leaves
Euphorbia antiquoranm	stems
Euphorbia acurcum	leaves, stems
Euphorbia hirta	plant material
Euphorbia hirta	stems
Euphorbia longana	leaves
Euphorbia neriifolia	leaves
Euphorbia neriifolia	stems
Festuca parrigluma	herb
Ficus bengalensis	leaves
Ficus nitida	leaves
Flotowia dicamthoides	leaves
Fluggea microcarpa	not stated++
Fluggea virosa	bark
Fluggea virosa	leaves
Frullania tamarisci	stems
Fucus evanescens	not stated++
Grewia asiatica	whole plant
Glochidio macrophyllun	stem bark
Glochidion puberum	leaves, stems

Table : 6 Continued

Name of Plant	Part used
Glycosmis mauruiana	leaves, stems
Gauzuma tomentosa	leaves, stems
Guazuma tomentosa	leaves
Gymnocolea inflata	whole plant
Gymnosporia wallichiana	whole plant
Haplopappus angustifolius	not stated++
Haplopappus foliosus	not stated++
Haplopappus heterophyllus	not stated++
Hemarthria sibirica	herb
Hemrocallis longituba	roots
Heterophragma adenophyllan	fruit
Holcus lanatus	herb
Holoptelea integrifolia	leaves
Hydnocarpus octandra	fruit, wood
Hydnocarpus octandra	bark
Hypserpa nirida	leaves
Inperata eylindrica	culms, blades
Imula helenium	oil from root
Iresine pringlci	ground stem, leaves
Kalanchoe sparhulata	flower
Kielmeyera rosea	branches
Leynephora moorei	whole plant
Lithocarpus attenuata	leaves
Lithocarpus attenuata	stems
Lithocarpus cornea	leaves, stems
Lithocarpus elieabethac	leaves, stems
Lithocarpus glagbra	leaves, stems
Lithoocarpus haipinii	leaves
Lithocarpus hdipinii	stems
Lithocarpus hancei	leaves
Lithocarpus hancei	stems
Lithocarpus harlandi	leaves
Lithocarpus harlandi	stems
Lithocarpus irwinii	leaves
Lithocarpus irwinii	stems
Lithocarpus litchioides	leaves
Lithocarpus litchioides	stems
Lithocarpus polystachra	leaves, stems
Lophatherum gracile	culms, blades
Lophopetahum rigidum	bark

Table : 6 Continued

Name of Plant	Part used
Macaranya kmarius	stem,
Madhuca butyracea	bark
Madhuca neriifolia	bark, wood
Mallorus paniculatus	leaves
Mangitera indica	roots
Mayetus neterophylla	bark
Melalenea leheudendron	leaves, stems
Melanolepis multiglandulosa	not stated++
Microstegium cimineum	herb
Mikania cordata	roots
Millettia dielsiana	leaves
Millettia nitida	leaves
Milleuia pachycarpa	leaves
Mimosa rubicaulis	roots
Miscamthus sachariflorus	culms, blades
Miscanthus sinensis	culms, blades
Monostroma nitidum	whole plant
Nephelium litchi	bark
Notonia grandiflora	leaves
Olearia poniculata	bark, roots
Olearia paniculata	leaves
Ophiorrhiza japonica	whole plant
Oplismensus undulatifolius	herb
Opuntia vulgaris	whole plant
Orixa juponica	leaves
Pachysandra terminalis	not stated++
Paederia foetida	not stated++
Pedilantus calcaratus	whole plant
Pedilanthus tehuacanus	whole plant
Pedilanthus tithymaloides	not stated++
Phacehurus latifolius	culms, blades
Photina glabra	leaves
Phyllanthus reticulatus	leaves
Phyllanthus reticulatus	stems
Phyllostachys heterocycla	culms way
Pinus roxburghi	bark
Pinus serotina	bark
Piper aurantiacum	seeds
Pleurostylia africana	leaves
Poa annua	herb

Table : 6 Continued

Name of Plant	Part used
oa sphondylodes	herb
Polygonum plebejum	flowers
Prunus eusitanica	leaves
Prunus nepalensis	bark
Prunus turfosa	bark
Ptrospermum acerifolitan	flowers
Panica granatum	bark
Putranjira roxburghii	bark
Putranjira roxburghii	leaves
Putranjira roxburghii	root bark
Pyrus communis	bark
Pyrus lanata	leaves, bark
Pyrus malus	bark
Pyrus pashia	bark, leaves
Pyrus pashia	stems
Pyrus sikkimensis	whole plant
Quercus	bark
Quercus acuta	leaves
Quercus acutissima	pollen
Quercus bambusaefolia	leaves
Quercus bambusaefolia	stems
Quercus championi	leaves
Quercus championi	stems
Quercus gilca	leaves
Quercus glauca	leaves
Quercus ilex	heart wood
Quercus incana	stem bark
Quercus lanceaefolia	stem bark
Quercus myrsinaefolia	leaves
Quercus myrsinaeefolia	stemp
Quercus pachyphylla	plant material
Quercus petracea	bark
Quercus phillyracoides	leaves
Quercus robur	bark
Quercus semicarpifolia	bark
Quercus sessilifolia	leaves
Quercus stenophylla	leaves, branches
Quercus suber	cork wax
Quercus suber	see also cork
Roupellia grata	whole plant
Rhododendron adamsii	defoliated shoots

Table : 6 Continued

Name of Plant	Part used
Rhododendron arboreum	leaves
Rhododendron aureum	defoliated shoots
Rhododendron barbatum	leaves
Rhododendron campanulatum	leaves
Rhododendron championae	leaves
Rhododendron ciunamomeum	leaves
Rhododendron colletianum	not stated++
Rhododendron cucasicum	stemp, leaves
Rhododendron dahuricum	defoliated shoots
Rhododendron decipiens	leaves
Rhododendron degronianum	leaves
Rhododendron falconeri	leaves
Rhododendron ferrugineum	not stated++
Rhhododendron grande	bark, leaves
Rhododendron hodgsonii	not stated++
Rhododendron kotschyi	defoliated shoots
Rhododendron luteum	defoliated shoots
Rhododendron metternichil	leaves
Rhododendron mucronulatum	defoliated shoots
Rhododendron nilagiricum	leaves
Rhododendron niloeum	leaves
Rhododendron ponticum	leaves
Rhododendron reticulatum	leaves
Rhododendron schlippenbachii	defoliated shoots
Rhododedron westlandii	not stated++
Rhodomyrtus tomentosa	not stated++
Saccharum spontancum	culms, blades
Salacia fruiticosa	root bark
Salacia prinoides	root bark
Salix japonica	bark
Salria glutinosa	gum exudate
Sapium sebiferum	leaves
Sarcostemma viminale	whole plant
Saussurea lappa	root oil
Scolapia crenata	not stated++
Scolapia schieberi	bark, wood
Secanone afzelli	roots
Sctaria chondrachne	grains
Sererinia buxifolia	leaves

Table : 6 Continued

Name of Plant	Part used
Siphonodon australe	bark
Sorghum japonicum	grains
Spatholobus roxburghii	heans
Spireae formasama	not stated++
Stachytarpheta indica	leaves
Stereocanlon paschale	whole plant
Syzygium cordatum	bark, sapwood
Talguena quinquenervis	not stated++
Tillandsia usneoides	whole plant
Treloa trinervis	not stated++
Trichadenia aeylanica	bark, wood
Tripetaleia paniculata	leaves, flowers
Tripetaleia paniculata	wood
Ulmus campestris	bark
Undaria pinnatifida	not stated++
Vaecinium bracteatum	flowers
Vaceinium bracteatum	leaves
Vaccinium membranaceum	roots
Vaccinium membranaceum	cork
Vaccinium uliginosum	leaves
Vanillosmopsis erythropappa	not stated++
Vincetoxicum	herb
Viola odorata	leaves
Viola odorata	wax
Vitex trifolia	leaves
Vittadinia australis	whole plant
Zea mays	herb
Zelkowa serrata	bark
Zoysia tenuifolia	herb
Coal	
Cork [presumably Quercus suber (389)]	

CHAPTER - 5

CHAPTER - 5

SUMMARY

Vitis Quadrangularis (Vitaceae) grows all over Bangladesh and also is cultivated as an ornamental plant in gardens. This plant also grows in other parts of Indian sub-continet[25]. It is a well known medicinal plant of country people. *Vitis quadrangularis* has earned good name and potential for the treatment of fractures of bones, eye diseases, chronic ulcers, piles & blindness. The juice of the stem is useful in scurvey and in irregular menstruation [21], asthma, indigestion, back pain and spine complaints.

A literature survey revealed that a lot of work have been carried out on many species of this genus and *Vitis qudrangularis* is a natural laboratory of many compounds such as, tetracyclic triterpenoids[36], onocer-7-ene-3 ∞ 21 ß - diol, ∞ - amyring, ∞ - amyron, sitosterol, ∆ - amyrin[41], Oxo - steroid, ß - sitosterol, carotenes, ascorbic acid, calcium oxalate [31] etc. But there appears no report of isolation of frieledin from *Cissus* or *Vitis quatrangularis* plants. This fact has prompted us to investigate this plants.

Vitis quadrangularis was collected from Barisal and Bangladesh University of Engineering and Technology Campus, Dhaka. The stem of the plant sliced in to small pieces and dried at a room temperature and extracted with MeOH, pet. ether and chloroform separately.

The extracts of *Vitis quadrangularis* yielded M_1, P_1 and C. Later on, M_1 were renamed as S_1 and P_1 renamed as S_2. Finally S_1 and S_2 were identified as triterpenoid, Friedelin, in both the extracts S_1 and S_2. Pure compound friedelin was isolated from both polar and non polar solvents.

The spectral techniques IR, ^{1}H - NMR, ^{13}C-NMR and GC-MS have been extensively used for the characterization of the compound.

Though only one compound "Friedelin" was isolated from the extracts. But the compound have great significance because of its medicinal importance. Friedelin has been considered[145], for treatment of "cancer of the bladder[123], convulsions, inflamnaton[124], topical ulcers, rheumatic inflammation, fever and dysentery [125]. Friedelin has also been found to have an "antifeedant" activity towards some insects.

Probably the existence of "Friedelin" in this plant has imparted all medicinal im portance so a brief review work was being deflected here on Friedelin.

CHAPTER - 6

CHAPTER - 6

REFERENCES

1. Korolkovans A. and Burckhalter, J. H. *Essentials of medicinal chemistry* (John willey and sons, New York, London) 14, 1996.

2. Liang Huang in, *Natural products and drug development*, Kroogasrd - larsen, P.S.B. christensen and H. Kofod; Eds; Munksgaard, Copenhagen; 94, 1984.

3. Kirtiker K.R. and Basu B.D. *Indian Medicinal plants*, (2nd edition, international book distributors publications, India), 3, 1710-1734, 1981.

4. Yusuf M.,Chowdhury Jasimuddin, Wahab M.A., Begum Jaripa; *Medicinal plants of Bangladesh*, BCSIR Chittagong, 1994.

5. Daustur J.F., *Mdeicinal plants of India and pakistan*, F. N.I.; 169-170, 1988.

6. Kirtiker K.R. and Basu B.D., *Indian Medicinal plants*, (2nd edition, published by B. singh and M.P Singh, New connaught place, Dehardun, India) Vol.-2, 1975.

7. Chowdhury A.K. A., Unpublished work.

8. Ali M. Erfan, Biswas K.M. and Hoque Eshanulb, *Indian Chem. J*; 15(3) 396, 1977.

9. Madar Z, Abel R., Samish S. and J. Ahad; Glucose lowering effect of fenugreek in non insulin dependent diabetes, Eur. Clin. Nutr.; 42, 51-54, 1988.

10. Chowdhury A.K. Azad, Khaleque R.A. and chakder S.K. Indian J. Med. Res., 80, 372, 1984.

11. Agarwal S.S, Chataku and Arora R.B.; Antifertility activity of the roots of Abrus pre-catonius linn., *Pharmacol. Res. Commun;* 2, 156, 1970.

12. Garg G.P. and Garg S.K.; Antifertility effect of *Areca catechu* and *Carica papaya* in female albino rats. *Indian j. Pharm;* 3, 23, 1971.

13. Chowdhury A.K.A.; Sushanta K.C. and Khan A.K.A., Antifertility activity of *Plumbago zeylanica* Linn. Roots, *Indian j. Med. Res.* 76,991, 1982.

14. Hecker E., Lutz D., Wever J., Goerltler K., Morton J.F., Proceeding of the XIIIth International Cancer Congress, 8-15, September 1982, Seattle Washington Alan, R. Liss New York, 1983.

15. Al-Yahya M. A., Phyto chemical studies of the plants used in traditional medicine of Saudi Arabia, *Fitoterapia,* 57 (3); 179-182,1986.

16. Kirtikar K. R. and Basu B. D., *Indian Medicinal Plants* (2nd addition, International book distributors publications, India), 1, 437, 1935.

17. Der-Marderosian A. H.; Pharmacogonosy, "Medicinal Teas Boon or Bane *"Drug Ther,* 7, 178-186, 1977.

18. Chopra R. N., Chopra I. C., Elunda K. L. and Kapur L. D., *Chopras indigenous drugs of India,* 510, 1958, U. N. Dhur and sons Private Ltd.

19. Kirtikar K. R. and Basu B. D., *Indian Medicinal Plants,* published by Sudhinar Basu, Bhuwaneswari, Asrama, Bahadurzon; Allahabad, India, Part-II, 865, 1918.

20. The wealth of *India - Dictionary of raw materials and industrial products,* CSIR, New Delhi, Dept. of Sci, Res. Govt. of India, Vol.-VI, 1962:

21. *Indian Medicinal plants*, Ltd. Colonel K.R. KIRTIKAR, F. L. S., I. M. S (Retired), Major B.D. Basu, M.R.C.S. (Eng.) I.M.S. (Retired), and an I.C.S. (Retired) 1993.

22. *The Flora of the plani Hills*, South India. K. M. Mathhew 1999. The Rapinat Herbarium, St Joseph's college.

23. *Flowering plants of the world* consultant editor : V. H. Heywood, oxford. Advisory editor : Professor D.M. Moore Phd FLS, I.B.K. Richardson B.Sc., Professor W.T. stearn Hon DSc Leiden, Hon ScD Cambridge, Hon Dphil Uppsala. FLSFI Biol, 1993. Vol-1.

24. Bhutani, K.K. etal., *phytochemistry*, 1984, 23, 407.

25. *The wealth of India (Raw Materials)* 1950, Vol. 2. P.184 CSIR, New Delhi, India.

26. Satyavati, G. V. (1976) *Medicinal Plants of India*, Vol. 1. P. 242.

27. Md. A. Hasan, *Local Medicinal Plants in Bangladesh* Dhaka University. Published - Feb. 1998. P. 90-93.

28. Yoshiteru Oshima AKio Kamijou, Hiroshi Mortani, Kei-ichi Namao, and Yasushi Ohizumi pharmaceutical Institute, Tohoku University, Aobayama, Aoba-ku, *sendai* 980, Japan. 1993, 58, 850.

29. Karl. C. et al., *Z Naturforsch.*, C, 1983, 38, 13 (isol).

30. Brieskorn, carl Heinz ; Blosczyk, Guenter (Inst. pharm. Lebensmittelchem., Univ, Wuerzburg, Fed. Rep. Ger.) Z. Lebensm- Unters. Forsch-Forsch. 1981, 173 (2), 201-5 (Ger).

31. Abdul Ghani, *Medicinal plants of Bangladesh (Chemical constituents and uses).* Jahangir Nagar University, Bangladesh, Published - March, 1998, P-129.

32. Major B. D. Basue. IMS (etired), *Indian Medicinal Plants*, Plate No. 24. Published 1980, Publisher's Sudhindra Nath Basu, panini, Elahabad.

33. Chopra, R.N. Nayar, S.L., Chopra, I.C. *Glossary of Indian Medicinal plants*, 1956, P. 24-5, CSIR, New Delhi, India.

34. Oshima, Y. et al., *Tetrahedron*, 1995, 51, 11974. (isol, uv, ir, pmr, cmr, ms).

35. Quei Index. *Name chages in Bangladesh Angiosperm* - A. M. Huq. principal scientific officer, Bangladesh National Herbarium, published 1986.

36. Madan, M. Gupta and Ram K. Verma, *Central Institute of Medicinal and Aromatic plants*, Lucknow 226016, India, 1990, 29, 336 (isol, pmr, ms).

37. (a) Kiso, Y.; Tohkin, M. ; Hikino, H. *Planta Medica* 1983, 49, 222-225.
(b) Kiso, Y. ; Tohkin, M. ; Hikino, H. *J. Nat. Prod.* 1983, 46, 841-847.
(c) Yang, L.-L. ; Yen, K. - Y. ; Kiso, Y. ; Hikino, H. *J. Ethnopharmacol.* 1987, 19,103-110.

38. Oshima, Y. ; ueno, Y. ; Hikino, H. ; Yang, L.-L. ; yen, K.-Y. *Tetrahedron* 1990, 46, 5121 - 5126.

39. Langcake, P.L. ; Pryce, R.J. Experientia 1977, 33, 151 - 152.

40. Karmen, A. ; Wroblewshi, F. ; Ladue, J. J. Clin. Invest. 1955. 34, 126 - 133.

41. Investigation of Medicinal Plants part II. Bhutani K.K. Kapoor, R. ; Atal, C.K. (Reg. Res. Lab. CSIC, Jammu - Tawi, 180001 India). *Phytochemicstry* 1994, 23 (2). 407 - 10 (Eng.).

42. J. S. Mathews, *Biochem ET Biophys Acta,* 1963, 69, 163.

43. A. Hisham, G. Jaya kumar, Y. Fujmoto 1995, *Phytochemistry,* Vol. 40, P - 1227 - 31.

44. Akihisa, T., Yamamoto, K. ; Tmura. T., Kumura, Y., lida, T., Nambara, T. and chang,F.C. 1992, *Chemical and phyarmaceutical bullatine*, 40, 789.

45. Joy Klass, Winston F. Tinto, Stewart Mclean and William F. Reynolds.; *Journal of natureal products*, Vol. 55, No. - 11, P - 1626 - 1630.

46. Kulshreshtha, M. J., Kulshreshtha, D. K. and Rastogi, R.P. (1972) *Phytochemistry* 11, 2369.

47. Sainsbury, M. (1970) *Phytochemistry* 9, 2209.

48. Friedel, C. (1892) Bull. *Soc. Chim.* Fr. 7, 164.

49. Karrer, W. (1958) *Konstitution und Vorkommen der Organischen Pflanzenstoffe*, Birkhauser, Basel.

50. (1940) *Elsevier's Encyclopedia of Organic Chemistry*; Vol. 14, P. 588; (1952) suppl. Vol. 14S, p. 825. Elsevier, New York.

51. Chevreul, M. (1815) Ann. Chim. 96. 141.

52. Istrati, C. and Ostrogovich, A. (1899) C. R. 128, 1581.

53. Drake, N. L. and Jacobsen, R. P. (1935) *J. Am. Chem. Soc.* 57, 1570.

54. Drake, N. L. and Shrader, S. A. (1935) *J. Am. Chem. Soc.* 57, 1854.

55. Drake, N. L. and Haskins. W. T. (1936) *J. Am. Chem.* Soc. 58, 1684.

56. Drake, N. L. and Campbell, W.P. (1936) *J. Am. Chem. Soc.* 58, 1681.

57. Drake, N. L. and Wolfe, J. K. (1939) *J. Am. Chem. Soc.* 61, 3074.

58. Seoane, E. (1957) *Rev. Cienc. Apl. 11.* 393 (Chem. Abstr. 52, 9039b).

59. Drake, N. L. and Wolfe, J. K (1940) *J. Am. Chem. Soc.* 62, 3018.

60. Lander, J. J. and Svirbely, . J. (1944) *J. Am. Chem. Soc.* 66, 235.

61. Ruzicka, L., Jeger, O and Ringnes. P. (1944) *Helv, Chim.* Acta 27,972

62. Perold, G. W., Meyerhans, K., Jeger, O. and Ruzicka. L. (1949) *Helv.Chim. Acta* 32, 1246.

63. Dutler, H., Jeger, O. and Ruzicka. L. (1955) Helv. *Chem Acta* 38, 1269.

64. Corey, E. J. and Ursprung, J. J. (1955) *J. Am. Chem. Soc.* 77, 3668.

65. Corey, E. J. and Upsprung, J. J. (1956) *J. Am. Chem. Soc.* 78, 5041.

66. Brownlie, G. Spring. F. S., Stevenson, R. and Strachan. W. S. (1956) *Chem. Ind. (London)* 686.

67. Brownlie, G. Spring. F. S., Stevenson, R. and Strachan. W. S. (1955) *Chem. Ind. (London)* 1156.

68. Brownlie, G., Spring. F. S., Stevenson, R. and Strachan. W. S. (1956) *J. Chem. Soc.* 2419.

69. Takahashi, T. and Ourisson. G. (1956) *Bull. Soc. Chim.* Fr. 353.

70. Coates, R. M. (1967) *Tetrahedron Letters* 4143.

71. Courtney, J. L. and Gascoigne, R. M. (1956) *J. Chem. Soc.* 2115.

72. Corteny, J. L., Gascoigne, R. M. and Szumer, A. Z. (1956) *J. Chem. Soc.* 2119.

73. Courtney, J. L., Gascoigne, R. M. and Szumer, A. Z. (1956) *Chem. Ind. (London)* 1479.

74. Courtney, J. L., Gascoigne, R. M. and Szumer, A. Z. (1958) *J. Chem. Soc.* 881.

75. Djerasi, C., Riniker, R. and Riniker, B. (1956) *J. Am. Chem. Soc.* 78, 6362.

76. Djerassi, C. and Klyne, W. (1957) *J. Am. Chem. Soc.* 79, 1506.

77. Rogers, D. and Thomas, D. V. (1956) *Bull. Soc. Chm.* Fr. 361.

78. Masaki, N., Niwa, M. and Kikuchi, T. (1975) *J. Chem. Soc. Perkin Trans.* 2, 610.

79. Laing, M., Burke-Laing, M. E., Bartho, R. and Weeks, C. M. (1977) *Tetrahedron Letters* 3839.

80. Stevenson, R. (1961) *J. Org. Chem.* 26, 2142.

81. Kane, V. V. and Stevenson, R. (1960) *J. Org. Chem.* 25, 1394.

82. Cambie, R. C. and Parnell, *J. C.* (1969) *N. Z. J. Sci.* 12, 453.

83. Sengupta, P. and Mukherjee, *J.* (1969) *Tetrahedron* 24, 6259.

84. Murakami, T., Itokawa, H., Uzuki, F. and Sawada. N. (1965) *Chem. Pharm. Bull.* 13, 1346.

85. Ikan, R., Kashman, J. and Bergmann, E. D. (1964) *J. Chronatogr.* 14, 275.

86. Kaganovich, F. L. Belikevich. P. I. and Dolidovich, E. F. (1972) *Vestsi Akad. Navuk B. SSR, Khim.* Naruk 110 (Chem. Abstr, 78, 6393m).

87. Fischer, F. and Hertel, R. (1968) *J. Chromatogr.* 38, 274.

88. Barua, A. K., Dutta, S. P. and Pal. S. K. (1967) *J. Chromatogr.* 31.569.

89. Ourisson G. and Takahashi, T. (1955) *Chem. Ind. (London)* 1155.

90. Cole, A. R. H., Muller, G. T. A., Thornton, D. W. and Willix, R. L. S. (1959) *J. Chem. Soc.* 1218.

91. Oganesyan, E.T., Shinkarenka, A. L. and Bandyukova, V. A. (1968) *Khim, Prir, Soedin.* 4, 212 *(Chem. Abstr.* 70, 58056j).

92. Brooks. C. J. W., Eglinton. G. and Hanaineh. L. (1966) *Spectrochim. Acta* 22, 131.

93. Djerassi, C., Budzikiewicz, H, and Wilson. J. M. (1962) *Tetrahedron Letters* 263.

94. Budzikiewicz, H, Wilson, J. M. and Djerassi, C. (1963) *J. Am. Chem. Soc.* 85, 3688.

95. Budzikiewicz, H. Brauman, J. I. and Djerassi C. (1965) *Tetrahedron letters 21*, 1855.

96. Courtney, J. L. and Shannon J. S. (1963) *Tetrahedron letters* 13.

97. Shannon, J. S. Macdonald. C. G. and Courtney. J. L. (1963) *Tetrahedron Letters* 173.

98. Huneck, S. and Tummler. R. (1968) *J. Prakt, Chem.* 38, 233.

99. Hirota, H., Moriyama, Y., Tsuyuki, T., Tanahashi, Y. Takahashi, T., Katoh, Y. and Satoh, H. (1975) *Bull. Chem. Soc. Jpn.* 48, 1884.

100. Shamma, M., Glick. R. E. and Mumma, R. O. (1962) *J. Org. Chem.* 27, 4512.

101. Wilson, R. G. and Williams, D. H. (1969) *Tetrahedron* 25, 155.

102. Demarco, P. V., Elzey. T. K., Lewis. R. B. and Wenkert E. (1970) *J. Am. Chem. Soc.* 92, 5737.

103. Buckley, D. G., Green, G. H., Ritchie, E. and Taylor, W. C. (1971) *Chem. Ind.* (London) 298.

104. Wenkert, E., Clouse, A. O., Cochran, D. W. and Doddrell, D. (1969) *J. Am. Chem. Soc.* 91,6879.

105. Beierbeck, H. and Saunders, J. K. (1975) *Can. J. Chem.* 53, 1307.

106. Beierbeck, H., Saunders, J. K. and ApSimon, *J. W.* (1977) *Can, J. Chem.* 55, 2813.

107. Kikuchi, T., Shingu, T., Niwa, M. and Yokoi, T. (1973) *Chem. Pharm. Bull.* 21, 1396.

108. Kikuchi, T., Niwa, M., Takayama, M., Yokoi, T. and Shingu, T. (1973) *Tetrahedron Letter* 1987.

109. kikuchi, T. and Toyoda, T. (1967) *Tetrahedron Letters* 3181.

110. Kane, V. V. and Stevenson. R. (1960) *Chem, Ind* (London) 1243

111. Tsuyuki, T. Tanahashi, Y., Sudo, Y., Nakamata, S. and Takahashi, T. (1966) *Bull. Chem. Soc.* Jpn. 39, 1610.

112. Shoppe, C. W. and Johnston G. A. R. (1962) *J. Chem. Soc.* 1246.

113. Kohen, F. and Stevenson. R. (1966) *Chem. Ind. (London)* 1844.

114. Drefahl, G. and Huneck, S. (1962) *J. Prakt. Chem.* 19, 14 *(Chem. Abstr.* 58, 692.

115. Witz P., Herrmann, H., Lehn, J. M. and Ourisson, G. (1963) *Bull. Soc. Chem. Fr.* 1101.

116. Sengupta. P., Mukherjee, J. and Sen, M. (1971) *Tetrahedron.* Soc. 27, 2473.

117. Rees. H. W. and Goodwin, T. W. (1972) in Biosynthesis (Geissman, T. A., ed.) Vol. I. p. 59. *The Chemical Society, London.*

118. Sengupta, P. (1968) *Bull. Natl. Inst. Sci, India* 37,1.

119. Agata, I., Corey, E.J., Hortmann. A. G., Klein, J., Proskow, S. and Ursprung. J. J. (1965) *J. Org. Chem.* 30, 1698.

120. ApSimon, J. W., Baker, P., Buccini, J., Hooper, J. W. and Macaulay, S. (1972) *Can. J. Chem.* 50, 1944.

121. Evans. D. A. (1968) *Diss. Abstr.* B.29, 936.

122. lreland, R. E. and Walba. D. M. (1976) *Tetrahedron Letter* 1071.

123. Simon, H. C. (1976) Ger Offen. 2,508.338 (ClA61k) 19 Feb, 1976 (*Chem. Abstr.* 84, 169664q).

124. Chaturvedi, A. K,. Parmar, S. S., Bhatnagar, S. C., Mistra G. and Nigam, S. K. (1974) Res. *Commun. Chem. Pathol Pharmacol.* 9, 11.

125. Subramanian, S. S., Nair, A. G. R. and Vedanthan T. N. C. (1974) *Indian J. Pharm.* 36,15.

126. Abbassy, M. A., El-Shazli, A. and El-Gayar, F. (1977) *Z. Angew. Entomol,* 83,317 (*Chem. Abstr.* 87, 147016q).

127. Tsuda, K. and Sakai, K. (1960) *Chem. Pharm. Bull* 8,554.

128. Ikekawa, N., Morisaki, M. and Hirayama, K. (1972) *Phytochemistry* 11, 2317.

129. Bruun, T. (1954) *Acta Chem. Scand* 8,71.

130. Bruun, T. (1969) *Acta Chem. Scand* 23, 3038.

131. Djerassi, C. and McCrindle, R. (1962) *J. Chem. Soc.* 4034

132. Huneck, S. and Overton, K. H. (1971) *Phytochemistry* 10, 3279.

133. Ikan, R. and Kashman J. (1963) *Isr. J. Chem.* 1, 502.

134. McLean, J., Rettie, G. H. and Spring. F. S. (1958) *Chem. Ind.* (London) 1515.

135. Golovanov. N. G. (1969) *Priroda* (Moscow) 59 (*Chem. Abstro.* 72, 5011a).

136. Jarolim, V., streibl, M., Hejno, K. and Sorm, F. (1961) *Collect, Czech. Chem. Commun.* 26, 451.

137. Jarolim, V., Streibl, M., Horak, M. and Sorm, F. (1958) *Chem. Ind.* (London) 1142.

138. Golovanov, N. G., Borisoglebskii, V. V., Kuzmenko, E. A. Mazyra, L. N. (1967) Khim. Tverd. Topl. 3 (*Chem. Absts* 68, 1477s).

139. Ikan, R. and McLeap, J. (1960) *J. Chem. Soc.* 893.

140. Golovanov, N. G., Borisoglebskii, V. V., Kuzmenko, E. A. and Mazyra, L. N. (1968) *Khim. Tverd. Topl.* 57 (*Chem. Abstr.* 69, 37760y).

141. Golovanov, N. G., Borisoglebskii, V. V., Kuzmenka, E. A. and Mazyra, L. N. (1967) *Kuli.- Bytovye Izdeays* 82 (*Chem. Abstr.* 69, 37760y).

142. Ruzika, L. and Prelog, V. (1943) *Helv. Chim. Acta* 20, 975.

143. Amel, A., Akhtar, R. and Quereshi, A. H. (1971) *Pak. J. Sci. Ind. Res.* 14, 71.

144. Meyer, A., Jeger, O. and Ruzicka, L. (1950) *Helv. Chim. Acta* 33, 672.

145. Friedelen and associated triterpenoids, Chandler, R. Frank ; Hooper, Shiirely N. (coll. Pharm. Dalhousie Univ., Halifax, NS can.) *Phytochemistry* 1979, 18 (5), 711-24 (Eng.).