Chemical Investigation on the leaves of *Dillenia Pentagyna* (Hargeza)

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AND TECHNOLOGY (BUET), DHAKA
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Bangladesh University of Engineering and Technology, Dhaka
Department of Chemistry

Certification of Thesis

A thesis on

Chemical Investigation on the leaves of *Dillenia pentagyna* (Hargeza)

By

Abdul Hannan Khan

Has been accepted as satisfactory in partial fulfillment of the requirements for the degree of Master of Philosophy (M. Phil) in Chemistry and certify that the student has demonstrated a satisfactory knowledge of the field covered by this thesis in an oral examination held on May 28, 2006

Board of Examiners

1. *Dr. Shakila Rahman*
   Associate Professor
   Department of Chemistry,
   BUET, Dhaka
   — *Supervisor & Chairman*

2. *Prof. Nilufar Nahar*
   Department of Chemistry
   Dhaka University, Dhaka
   — *Co-Supervisor & Member*

3. *Dr. Nazrul Islam*
   Prof and Head
   Department of Chemistry,
   BUET, Dhaka
   — 28.5.06
   Member

4. *Dr. Enamul Huq*
   Professor
   Department of Chemistry,
   BUET, Dhaka
   — *Member*

5. *Prof. Md. Mosihuzzaman*
   Department of Chemistry
   Dhaka University, Dhaka
   — *Member (External)*

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This is to certify that the research work embodying in this thesis has been carried out under my supervision. The work presented herein is original. This thesis has not been submitted elsewhere for the award of any other degree or diploma in any University or institution.

Dr. Shakila Rahman
(Supervisor)
Associate Professor
Department of Chemistry
BUET, Dhaka
Bangladesh

DECLARATION

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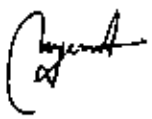

Abdul Hannan Khan
(Candidate)
M. Phil Student
Roll No. 100103107F
Department of Chemistry
BUET, Dhaka
Bangladesh

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Abdul Hannan Khan

(Author)
Department of Chemistry
Bangladesh University of Engineering and Technology (BUET)
Dhaka,
Bangladesh
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CHAPTER 1

1.0 INTRODUCTION

1.1 General:

Among all the creation of Allah human being and nature are interdependent. Plant kingdom has supported to safeguard the survival of the human being on earth from the emergence of the civilization. In fact a major part of the four basic needs of human life that are food, cloth, medicine and shelter are obtained from plant kingdom. The raw materials for these items are directly and indirectly produced by the plants and are very seldom used by them but serve humanity in many ways. A major part of the global energy requirement is also supplied by the plant source as fuel.

Plants are important to human in many other ways. The role of forests and other types of natural vegetation in controlling foods and climate regulation are well known. Plant products have played an important role in the need of human being from the ancient time. The primary compound glucose and the by product oxygen which are most essential for our life are produced in plant from the simple compounds carbon dioxide and water in the presence of sunlight and chlorophyll by the process of photosynthesis. But this is not possible for animals. So, they depend on plants. Plant produce, through metabolic activities, not only food materials but also other substances, such as glycosides, steroids, alkaloids, resins, essential oils, tannins flavonoids, pigments etc. These are usually called secondary metabolites of plant kingdom.

Since disease, decay and death have always co-existed with life, the study of diseases and their treatment must also have been contemporaneous with the dawn of the human intellect. It is apparent that whatever progress science might have made in the field of medicine over the years plant still remain the primary source of supply of many important drugs used in modern medicine. Indeed, the potential of obtaining new drugs from plant sources is so great that thousands of substances of plant origin are now being studied for activity against such formidable foes as heart diseases, cancer, diabetes and AIDS.

This type of study is sure to bring fruitful results, because of the fact that the plant kingdom represents a virtually untapped reservoir of new chemical compounds and it has been estimated that only 5-15% of the approximately 2,50,000-5.00,000 species of higher plants which is more than 80,000 are medicinal has been investigated pharmacologically[1] Thus, these are considerable chances of finding new natural compounds with pharmacological activities. useful for the development of new drugs. Scientists are now working together to find out new drug for incurable diseases. Taxonomist, Chemist, Biochemist, Pharmacologist and Pharmacist are working under collaborative program for making a plant product (s) into a commercial drug.

1.2 Medicinal importance of plant materials:

Medicinal plants include plant materials such as foliage, bark, root, flowers, fruit, seeds and biochemical or physiological by-products which may be used as such or in other forms like extracts, infusions, decoctions, powders etc. and naturally available chemical compounds isolated from them to produce drugs for human and veterinary use. These plants are closely related to or are also among the group that produce essential oils, condiments, spices and other higher forms of plant life that exert specific influences on metabolism in general.

Medicinal plants are now increasingly being used in raw, semi-processed and polypharmaceutical form as medicine Eastern medicine, which is playing a significant role in providing relief to a great number of people in Asia and to some extent in the whole Mediterranean region. Its allied system i.e. "Herbalism" or " Physiotherapy" is also being practiced in one form or another around the globe from time immemorial.

Nature served as the source of all medicinal agents for human since the prehistoric days. Even today, natural products and their derivatives and analogs represent over 50% of all drugs in clinical use of which higher plant derived natural products accounting for ca 25% of the total[2]. The World Health Organization (WHO) estimates that 80% of the people in developing countries of the world rely on traditional medicine for their primary health needs, and about 85% of the

traditional medicine involves the use of plant extracts. This means that about 4 billion people in the world rely on plant as sources of drugs [3].

Plants are used as a means of treatment of diseases from the early days of civilization on Earth. It can be traced back over five millennia to documents of the early civilization in India[4]. The medicinal use of plants in the Indian subcontinent is found in the 'Rig Veda' (4500-16-BC), the Indo Aryans used the soma-plant (*Amanita muscaria*) as a medicinal agent[5].

Biologists and Chemists throughout the world have been working for finding out the active Principles of the plants used for a particular disease and to find out lead compounds, which may be developed for the treatment of various diseases. Extensive research has resulted in discovering many drugs from plant sources. Secondary metabolities like steroids, terpenoids, alkaloids, glycosides, volatile oils, flavonoids and vitamins etc., which are synthesized by plants, showed biological properties. Many bioactive compounds were isolated and are still commercially used for the treatment of various diseases.

Now that a good number of the plants used in indigenous remedies to treat or present livestock diseases have been classified both scientifically and ethno taxonomically. This is expected to make a substantial contribution to the field of ethno veterinary research and to development on Cameroon and elsewhere. Formal research in ethno veterinary medicine will no doubt help to confirm the claims made by ethno veterinarians with respect to the efficacy of ethno veterinary treatments by ethno veterinarians. The idea of using medicinal plants to treat livestock is not new. Many of the active ingredients in chemically manufactured drugs were originally derived from plant compounds (e.g. pyrethroids).

In the past two centuries, chemical investigation and purification of extracts of plants for their medicinal properties, based on pharmacological studies and those used as toxins and hunting poisons in their native habitats, have yielded a number of useful compounds which have proven[6-8] to be indispensable in the practice of

modern medicine. For instance, the cure alkaloids from South America Vines had long been used by native to make arrow poisons, and *African strophanthus* species and calabar beans yielded medicinally useful cardiac glycosides and physostigmine respectively, which were originally used as arrow and ordeal poisons in their native habitats.

Some active constituents[9] from plants:

Mineral salts:

The salts of potassium and calcium are especially important. Potassium salts possess diuretic properties, while calcium salts contribute to bone structure, to the regulation of the nervous system and to the resistance of the patient to infection. The salts of potassium are found in abundance in almost all plants and they are generally present in soluble form. Calcium salts are much less soluble and reach the body only in small amounts.

Silicic acid is also present in almost all plants and some contain large amounts. This acid acts as a strengthener of conjunctival tissues, especially of the lungs, thus providing some slight increase in resistance to pulmonary tuberculosis. A balanced diet provides sufficient intake of mineral salts. Those supplied by medicinal plants are associated with the other active principles and the medicinal action of such mineral constituents is of supplementary importance.

Organic Acids

Organic acids (malic, citric, tartaric, oxalic, etc.) are also common constituents of plants: they accumulate, for example, in fruits. They act in certain cases as mild laxative, especially tartaric acid and its salts.

Mucilage

Mucilage in plants has the property of swelling in water to produce viscous pollutions. It is this property that produces their physical laxative effect; water is retained in the intestine, which prevents the contents from hardening and on the

contrary, acts as a lubricant. At the same time the content of the intestine increases in volume, increasing its pressure on the walls of the organ and favoring peristaltic movement. In addition, the mucilage forms a protective coat on the mucosa so that irritants (such as acids, salts etc.) are unable to come in contact with inflamed of diseased tissues. For these reasons mucilaginous drugs are used as laxative and for the protection of the inflamed mucosa of the digestive tract.

Alkaloids

Alkaloids are nitrogenous compounds that have more or less marked action on the central nervous system and often on the peripheral nervous system. Some alkaloids are among the most powerful poisons known. Plants containing alkaloids are used in traditional medicine: for example, *Hyoscyamus niger* (*Ajwain khurasani*) is used as a liniment to relieve pain. *Atropa belladona* and Aconitum are well known plants containing alkaloids.

Alkaloids from *Rauwolfia serpentina* have attracted global attention. Today we have encyclopedic publications on alkaloids isolated from plants and classified into various potentially active pharmacological groups.

Glycosides

Glycosides are substances that are decomposed into a non-sugar part and one or several sugars when hydrolyzed by enzymes, by dilute acids or alkalis or by boiling. Their medicinal action is due to the non-sugar part of the molecules, which are chemically very diverse. The sugar part of the molecule generally influences the solubility in water and hence its absorption by the body. Glycosides present in digitalis, strophanthus, urginea and veratrum are very cardio-active and at the same time increase diuresis Some glycosides found in aloe, cascara, rheum and cassia species are powerful laxatives.

Saponin glycosides obtained from *glycyrrhiza*, *cinchona*, *aesculus*, *aralia*, *ginseng* have important medicinal uses. Some glycosides strengthen the blood capillaries and prevent the small cutaneous haemorrhages so frequent in the aged.

Saponins

Saponins are also glycosides. Their outstanding physical character is that their aqueous solutions froth greatly; this is the reason for their use as detergents and it explains their name. Large doses in the blood stream are dangerous and may prove fatal by dissolving the red bold corpuscles.

Tannins

Tannins have the property of precipitating proteins. They are widespread through the plant kingdom, as for example, in oak bark, walnut leaves, the willows, roses, acacia, bauhinia, pterocarpus, terminalia, potentilla species etc. in the free state and in large doses they irritate the mucosa; in small doses they precipitate small amounts of proteins in the cells of the mucosa which are thus rendered impermeable; other irritants are thus prevented prom penetrating to the deeper layers of damaged mucosa, hence healing is aided. This property also explains the use of tannins as antidiarrhoeal and in the treatment of certain burns.

Volatile oils or essences

The volatile oils occur most frequently in special glands, either within the tissues or on the epidermal surface. Their medicinal activity is very variable. Some act on the central nervous system e.g. anise oil (carminative) or oil of wormwood (stimulant). Many increase the secretion of gastric juices (saliva, stomach and intestinal juices, bile) and hence increase appetite. They aid digestion and regularize intestinal action. When placed on the mucosa, on wounds or even on intact skin they can increase the flow of blood. Especially of leucocytes (hyperaemia).

Some volatile oil containing plants e.g. Juniper and *Carum copticum* stimujlate secretion of urine; these are used to reduce accumulation of water dropsy in the body. Menthol is digestive and a local antiseptic. Aromatic herbs and essential oils have attracted much attention from the cosmetic, soap and flavouring industries in recent times.

Resins

Resins are secreted by special glands similar to those that produce volatile oils and frequently at the same time as these. They are not volatile; they are used as skin irritants and purgatives e.g. ipomoea, asafoetida, myrrh, podophyllum etc.

Bitter principles

Bitter principles do not comprise any one chemical group, for their only common property is their bitter taste. But this property is of therapeutic significance. Taken by mouth they increase the secretion of digestive juices, and so increase the appetite of the patient. Among the bitter drugs are artemisia, swertia, gentian root, centaury, margosa and fumaria.

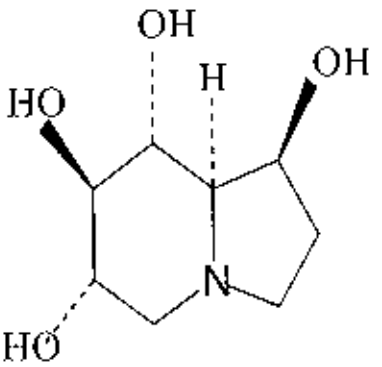
Antibiotics

They are extracted from fungi, the so-called lower plants (of which the best known is penicillin). They are of the utmost medicinal importance since they cure a number of infectious diseases.

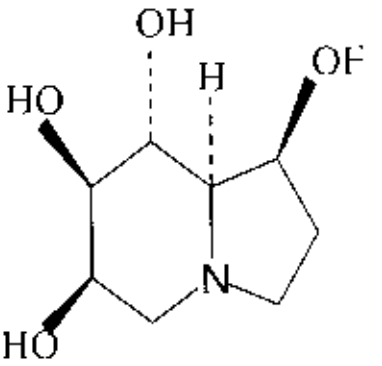
Although a majority of antibiotics are extracted from fungi, some antimicrobial activity has also been found in vertain higher (angiosperm) plants, which explains the anti infective action attributed to such plants.

Several species of a genus of gourds (Trichosanthes, Cucurbitaceae) widespread in Asia contain a toxic protein trichosanthin. Preparations based on this compound appear to have ribosome-inactivating properties and selectively kill cells infected with HIV[10]. Although the roots of T. kirilowii have traditional medicinal uses in China, Taiwan and Korea a number of AIDS suffers in the US who took this preparation on their own initiative suffered severe side effects, including death, illustrating the necessity for adequate testing of such materials.

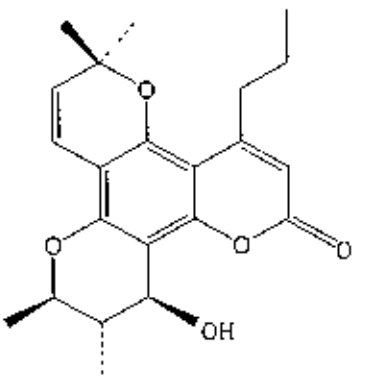
Most of the major chemical groups of natural products have yielded compounds with anti-HIV activity and in the reviews listed below over 80 such compounds are depicted: more continue to appear in the literature. As an illustration, a few plants and their active constituents are given in Table-(a) and formulae in fig below:



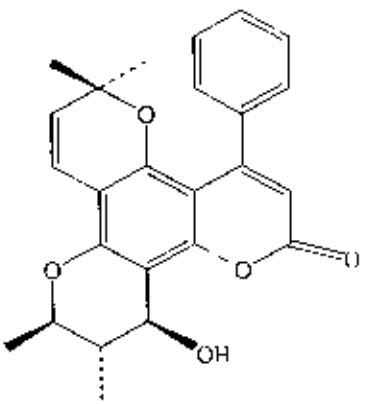
Castanospermine (1)



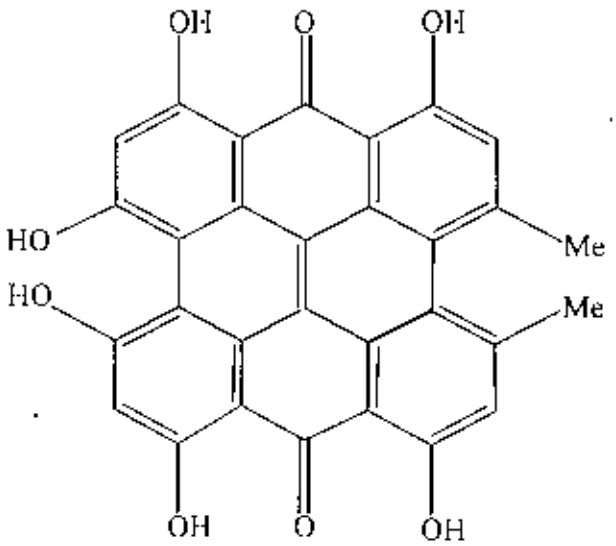
6-epi-Castanospermine (2)



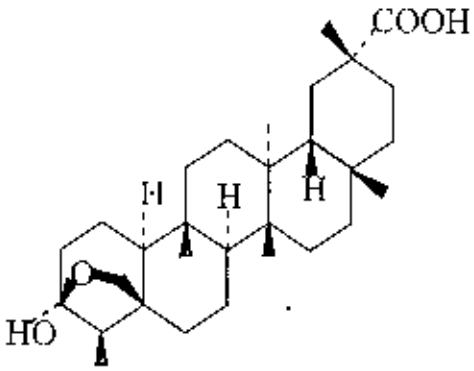
(+)-Calanolide A (3)



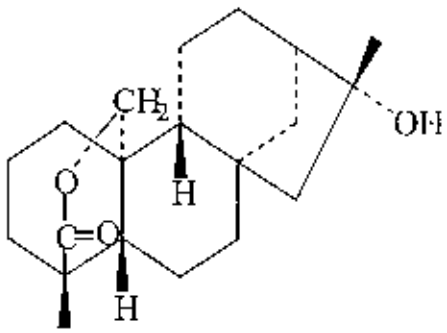
Inophyllum B (4)



Hypericin (5)



Salaspermic acid (6)



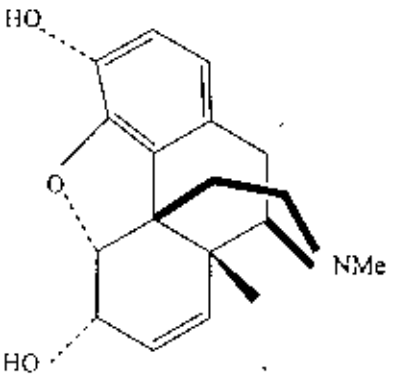
Tripterifordin (7)

Table-(a): Plant constituents with anti-HIV activity:

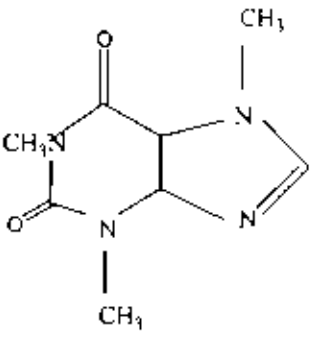
Constituents	Plant source	Observations
Alkaloids Castanospermine Michellamines A-C (Naphthylisoquinolin e dimmers)	Ancistrocladus korupensis (Ancistrocladaceae)	Michellamine B has broad range of anti-HIV activity.
Anthraquinones Hypericin	Hypericum spp. (Guttiferae)	Antiretroviral activity; clinical trials (1991)
Coumarins Lycopyranocoumarin Glycycoumarin	Glycyrrhiza glabra	Inhibition of giant cell formation in HIV-infected cell cultures
Dimeric sesquiterpenes Gossypol	Seeds of Gossypium spp	Inhibitory effect on HIV replication
Flavonoids Glycyrrhizoflavone Isolicoflavonol Licochalcone	Glycyrrhiza glabra	Similar action to liquorice coumarins
Lignans (-)-Trachelogenin	Ipomoea cairica (Convoolvulaceae)	Suppresses the integration of proviral DNA into cellular genome
Pentacyclic triterpenoids Glycyrrhizin Salaspermic acid	Slycyrrhiza glabra and lther spp.	Asymptomatic HIV carriers experienced delayed development of AIDS symptoms
Polysaccharides Sulphated polysaccharides	Various Chiness herbal medicines including Viola Yedoensis (Violaceae) Prunella vulgaris (Labiatae) Alternanthera philoxeroides (Amaranthaceae)	In vitro inhibitory activity against HIV
Tanins Tetragalloylquinic acids	Commercial tannic acid	HIV reverse transcriptase inhibitors

In 1804, the natural analgesic drug morphine (8) was isolated from latex of *Papever somniferum* capsules (opium) used for depressant action on various parts of the nervous system. The other important plant derived drugs have been discovered and introduced into modern science. Some examples of early drugs

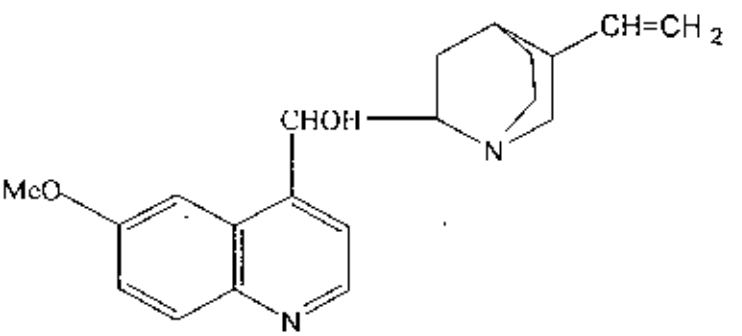
such as strychnine from *Strychnos nux vomica* (1817), emetine from *Cephaelis ipecacuanha* (1817), *caffeine* (9) from *Theasinensis* (1819) and quinine (10) from *Cinchona spp* (1820)[11].



Morphine (8)

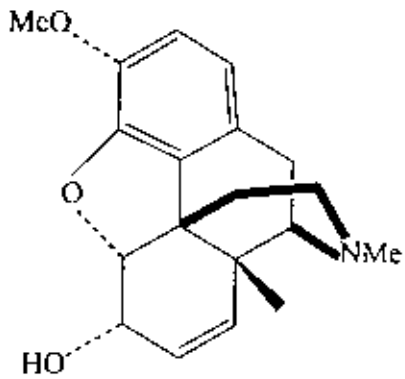


Caffeine (9)

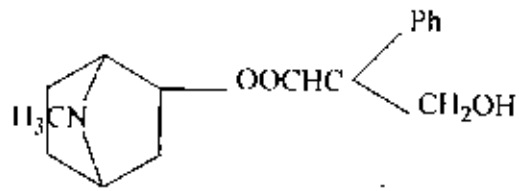


Quinine (10)

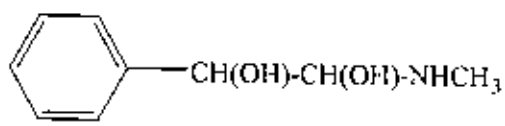
In this modern age, a number of bioactive alkaloids were isolated from poisonous plants based on folkloric history. Reserpine, used in western medinine as an antihypertensive and tranquilizer was isolated from the East Indian Snakeroot, *Rauvolfia serpentina* L. Benth. Ex Kurz, has been used for centuries as a native Kast Indian medicinal plant. Codeine (11), atropine (12), ephedrine (13), cocaine (14), theophyline (15), scopolamine (16), l-hyoscyamine, *pilocarpine*, vincristine, vinblastine etc. are good examples of useful modern drugs [12,13] isolated from poisonous medicinal plants.



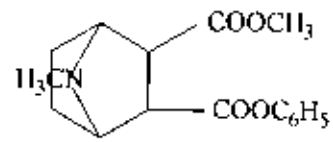
Codeine (11)



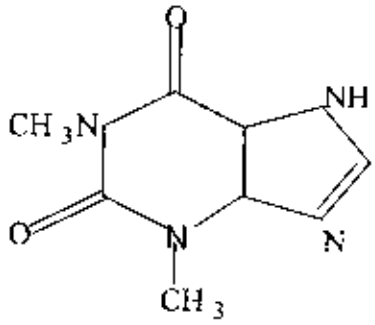
Atrophine (12)



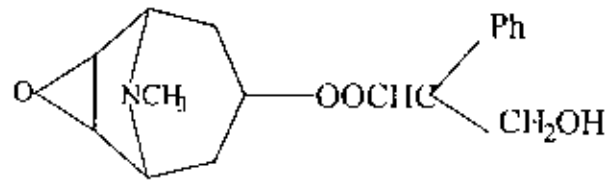
Ephedrine (13)



Cocaine (14)

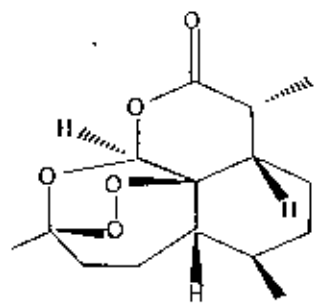


Theophylline (15)

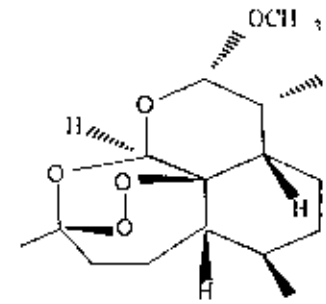


Scopolamine (16)

The new antimalarial compound artemisinin (17) has been isolated from the *Artemisia annua* herb. Ancient Chinese medicinal texts written two thousand years ago mentioned that the herb was used as a remedy for malaria. Artemether (18) a synthetic analogue of artemisinin has been developed in the People's Republic of China as an antimalarial agent[14]. A novel cumarin derivative calanolide A (3) is a reverse – transcriptase inhibitror discovered from the Malaysian rainforest tree *Calophyllum lanigerum* by the U.S. National Cancer Institute. It exhibits synergistic anti-HIV activity in combination with nucleoside reverse transcripts inhibitors.



Artemidinin (17)

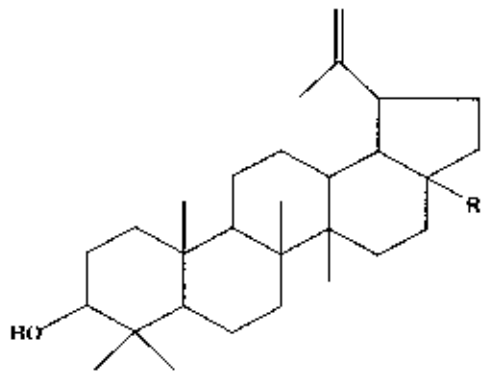


Artemether (18)

At least two thousand years ago tree had been known for its toxic properties. Thus, Julius Caesar recorded that Catuvolcus, king of the Eburenes, poisoned himself with yew rather than face capture by Caesar's legions[15]. Many other early accounts of yew poisoning have been reported [16]. Because of this, initial chemical

studies of the constituents of yew concentrated primarily on its toxic principles, culminating in the structure elucidation of the first taxane diterpenoids in the 1960's and taxol, an anticancer taxane diterpenoid derived from the yew tree, *Taxus brevifolia*, in 1992, which has been approved[17] for the treatment of refractory ovarian cancer. It is this, ironic that the yew, long known as a tree of death, showed the source of one of the most promising and important new anticancer drugs of the last twenty years.

In 1996, N. Nahar et.al. isolated four terpenoids namely lupeol (19), betulin (20), betulinaldehyde (21) and betulinic acid (22) from the bark of *Zizyphus rugosa* and *Zizyphus oenoplia*[18].



(19) R = Me, lupeol

(20) R = CH_2OH, betulin

(21) R = -CHO, betulinaldehyde

(22) R = -COOH, betulinic acid

Mamun et.al. (2001) studied the effect of *Pterospermum acerifolium* and *Pterospermum semisagittatum* as normal, Type I and Type II diabetic model rats[19]. The methanol extract of bark of *P. acerifolium* showed a significant antihyperglycemic effect in monodiabetic ($p<0.01$), Type II ($p<0.05$) and Type I

($p<0.01$) diabetic model rats. Methanol extract of leaves of *P.semisagittatum* showed significant lowering of serum glucose in normal ($p<0.05$-0.01), Type I ($p<0.05$) and Type II ($p<0.05$) diabetic model rats. Chloroform extract of leaves of *P acerifolium* showed significant anti-hyperglycemic effect ($p<0.05$) on Type I diabetic model rats.

Mosihuzzaman et.al[20] showed that husk of *Plantago ovata*, leaves of *Gymnema sylvestre*, bulbs of *Allium sativum*, *Allium cepa* and *Spirulina platensis* (cyanobacteria) had hypoglycemic effect in Type I and Type II diabetic model rats when the samples were fed simultaneously with glucose load. These three plants did not show any significant hypoglycemic activity in any of the model rats under fasting condition. But with glucose load, *P pvata*, *G sylvestre* and *S. platensis* in Type I model rats and only with *S. Platensis* in Type II model rats.

The juice of *Momordica charantia* is applied found the eyes for the cure of night blindness[21] and juice of the *Momordica charantia* and its different lowered blood fraction glucose [22].

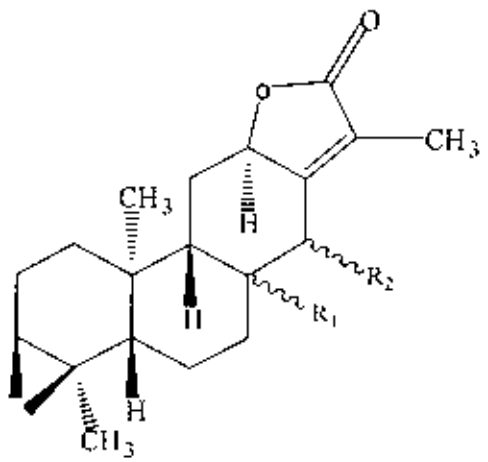
Rhizome of Costus specious tuber of *Nephrolepsis tuberosa* and bulb of *Stephania hernandifolia*, used by the local people and traditional healers in the Eastern Himalayan belt were studied [23] by Mosihuzzaman et.al. for their effects on serum glucose levels in nondiabetic and diabetic rat models at different prandial atates . However, when fed 30 men before the glucose load, both C. specious and *N tuberosa* showed hypoglycemic effect. In NIDDM model rats, *N. tuberosa* opposed rise in serum glucose level when it was fed 30 minutes before the glucose load, whereas *S. hernandifolia* had a tendency to raise the serum glucose level.

Trigonella foenumgraecum (methi) possesses tremendous importance as a medicinal shrub. One of the popular uses of *T foenumgraecum* is to reduce the blood glucose lebel of diabetic patients[24]. Hot water extract of acrial parts of *T. foenumgraecum* is used against sore throats.

The effects of orally administered extracts of *Coccinia indica* (leaves and steam), *Syzizum cumini* (seeds) and *Musa paradisiacal* (fruits) were investigated [25] in nondiabetic and streptozotocin (STZ) induced Type I and Type II diabetic model rats. The results revealed that *S cumini* had no significant on blood glucose levels in any model of rats. A consistent hypoglycemic activity was found with *C indica* and *M paradisiacal* powder and extracts in nondiabetic and Type II model rats when fed simultaneously with glucose load. The results show that *C. indica* and *M. paradisiacal* have hypoglycemic properties possibly medicated by enhanced disposal of glucose through increased insulin sensitivity. Taraxerol, stigmasterol, 3,4-dihydroxy benzoic acid and catechin isolated by N. Nahar et.al[26] from anti-HIV active and hypoglycemic extracts of *Fissistigma rubiginosum* plant. They also identified and quantified of phenolic acids in the leaves, bark and stem of the plant[27].

Dotriacontane, methyl octadecanoate and octacosanol-1 were isolated [28] from the chloroform extract *oxalis corniculata* Linn. Five phenolic aceds were identified and quantified in aqueous 80% ethanol extract and also three fatty acids were identified and quantified in the dichloromethane extract of this plant.

M. Iqbal choudhury[29] (2002) isolated two new novel diterpene lactones namely suregadolides A (23) and B (24) from a dichloromethane extract of *Suregada multiflora* bark. Suregadolides A (23) showed moderate inhibitory activity in a mutant yeast strain bioassay.



(23) $R_1 = R_2 = \beta$-OH Suregadolides A

(24) $R_1 = R_2 = \alpha$-OH Suregadolides B

Centella asiatica (Thankuni) has laxative, diuretic and antichloristic affects[30]. It acts as a tonic and blood purifier. It is also a household remedy[31] for skin diseases like chronic and obstinate eczema, chronic ulcers, syphilitic sores etc. The leaves of this plant are a household remedy for the treatment of early stage of dysentery in children.

Camptothecin (CPT. 11) has been isolated from *Camptotheca acuminata* tree native to China. Camptothecin was remarkably active in the life prolongation of mice treated with L1210 leukemia cells[32] showing activity in doses between 0.5 and 4.0-mg/kg body weights in this mouse life prolongation assay.

The commercial value of drug products still derived from higher plants is considerable and should not be underestimated. For example, in 1980 American consumers paid about 48 billion for prescription drugs derived solely from higher plant sources Table-(b).

Table-(b): Some example of economically important plant-derived drugs and intermediates that are obtained commercially from whole plant sources[33-36]

Compounds or Class	Plant sources	Therapeutic uses
A. Alkaloids: 1.Hemlock alkaloids (Coniine, γ-coniceine)	*Conium maculatum*	Paralyses the central nervous system
2.Piperine	*Piper nigrum*	Insenticide for houseflies.
3.Pelletierine	*Punica granarum*	Trejatment of tapeworm infections
4.Tobacco alkaloids (Nicotine)	*Nicotiana tobacum*	Increase the heartbeat. blood pressure is increased.
5.Tropane alkaloids (Atropine)	*Atropa belladonna*	Opthaquinology.
6.Coca alkaloids (Cocaine)	*Erythroxylon coca*	Local anaesthetic in eye surgery and dentistry.
7.Cinchona alkaloids (Quinine, cinchonine)	Cinchona spp.(Chinchona bark	Antimalarial
8.Opium alkaloids (Papaverine, morphine)	*Papaver somniferum*	Analgesecs, antitussive
9.Reserpine	*Rauvolfia serpentinal* L.Bentham ex Kurz.	Antihypertensive
10.Strychnine alkaloids (Strychnine and brucine)	*Strychnos ignatii*	Antidote
11.Colchicum alkaloids (Colchicines)	*Colchicum autumnale*	Diagonosis of Gout
12.Physostigmine	Physostigma venenosum	Cholinergic
13.Catharanthus (*Vinca*)	*Catharanthus roseus L.*	Anticancer
14 Taxol	*Taxus brevifolia Nutt*	Anticancer
B. Steroids: 1.Hormones Stigmasterol, (hecogenin) 2.Digitalis glycosides (Digoxin, digitoxin, gitoxin)	Dioscorea spp. (Mexican/yams);Soyabean-derived stigmasterol , *Digitalis purpurea* L., D. lanata	Oral contraceptives and other steroid drugs and hormones. Cardiotonic glycosides (cardenolides)

Plants have medicinal, herbal, cosmetics[9-10 37] and therapeutics uses too. A list of plants which are used as herbal remedy are sighted below:

Sl.No.	Name of Diseases	Plants
1.	Acne	Aloe Vera, Calendula, Cornflower, Feverfew, Goldenseal, Green Tea, Lemongrass, Violet
2.	Age Spots	Onion
3.	After-Shave	Sage
4.	AIDS	Aloe Vera, Green Tea
5.	Alertness	Ginkgo
6.	Allergic Reactions	Aloe Vera
7.	Allergies	Ginkgo
8.	Alzheimer's Disease	Broccoli, Ginkgo
9	Antiseptic	Cornflower, St. John's Wort
10.	Anti-Aging	Broccoli, Ginkgo, Ginseng, Green Tea
11.	Anti-Bacterial	Green Tea
12.	Antiperspirant	Sage
13.	Anti-Viral	Aloe Vera, Green Tea, St. John's Wort
14.	Anxiety	St. John's Wort, Valerian
15.	Aphrodesiac	Ginkgo, Ginseng
16.	Appetite Stimulant	Mint, Tarragon, Thyme
17.	Appetite Suppressant	Beans, Fennel
18.	Aromatherapy	Angelica, Lemongrass
19.	Arterial Disease	Ginkgo
20.	Arthritis	Aloe Vera, Angelica, Broccoli, Feverfew, St. John's Wort
21	Asthma	Aloe Vera, Chamomile, Feverfew, Ginkgo, Marjoram, Valerian
22.	Astringent	Scented Geranium
23.	Atherosclerosis	Ginkgo
24.	Athlete's Foot	Aloe Vera, Calendula, Garlic, Goldenseal, Lemongrass, Thyme
25.	Attention Deficit Disorder	Valerian
26.	Attention Span (increase)	Ginkgo
27.	Back Pain	St. John's Wort
28.	Bacterial (Anti)	Lemon Balm
29.	Bedwetting	Valerian
30	Bee Stings	Calendula, Parsley, Savory (Summer)
31.	Bladder Infections	Garlic, Parsley
32.	Blisters	Aloe Vera, Calendula
33.	Bloating	Oregano
34.	Blood Circulation (increase)	Ginkgo
35.	Blood Pressure (lower)	Chervil, Chives, Garlic, Green Tea, Onion,

		Tomato, Valerian
36	Blood Pressure (regulate)	Ginseng, Green Tea
37.	Blood Purifier	Echinacea
38	Blood Sugar Normalizer	Aloe Vera, Beans, Ginseng, Green Tea
39.	Blood Thinner	Garlic
40.	Bowels - Inflammatory Disease	Green Tea
41.	Breath Freshener	Anise, Fennel, Parsley
42.	Breast - Increase Mother's Milk	Dill, Fennel
43.	Acne	Aloe Vera, Calendula, Cornflower, Feverfew, Goldenseal, Green Tea, Lemongrass, Violet
44.	Age Spots	Onion
45.	After-Shave	Sage
46.	AIDS	Aloe Vera, Green Tea
47.	Alertness	Ginkgo
48.	Allergic Reactions	Aloe Vera
49.	Allergies	Ginkgo
50.	Alzheimer's Disease	Broccoli, Ginkgo
51.	Antiseptic	Cornflower, St. John's Wort
52.	Anti-Aging	Broccoli, Ginkgo, Ginseng, Green Tea
53.	Anti-Bacterial	Green Tea
54.	Antiperspirant	Sage
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72.	Bee Stings	Calendula, Parsley, Savory (Summer)
73.	Bladder Infections	Garlic, Parsley

74.	Blisters	Aloe Vera, Calendula
75.	Bloating	Oregano
76.	Blood Circulation (increase)	Ginkgo
77.	Blood Pressure (lower)	Chervil, Chives, Garlic, Green Tea, Onion, Tomato, Valerian
78.	Blood Pressure (regulate)	Ginseng, Green Tea
79.	Blood Purifier	Echinacea
80.	Blood Sugar Normalizer	Aloe Vera, Beans, Ginseng, Green Tea
81.	Blood Thinner	Garlic
82.	Bowels - Inflammatory Disease	Green Tea
83.	Breath Freshener	Anise, Fennel, Parsley
84.	Breast - Increase Mother's Milk	Dill, Fennel
85.	Breast - Fullness while Nursing	Dill, Fennel
86.	Bronchitis	Borage, Hyssop, Chamomile, Oregano, St. John's Wort, Thyme, Violet
87.	Bruises	Bay, Borage, Calendula, Caraway, Cornflower, Feverfew, Hyssop, Sage, St. John's Wort, Rosemary
88.	Burns	Aloe Vera, Calendula, Chamomile, Echinacea, Onion, St. John's Wort
89	Cancer - Breast	Broccoli, Green Tea
90	Cancer - Colon	Broccoli
91.	Cancer - Esophageal	Green Tea
92.	Cancer - General	Aloe Vera
93.	Cancer - Liver	Green Tea
94.	Cancer - Lung	Aloe Vera, Broccoli
95.	Cancer - Pancreatic	Green Tea

Drugs acting[10] on mental activity, central nervous system, urinary and reproductive systems, skin and mucous membranes:

Drugs affecting mental activity	
Lysergic acid diethylamide	Hallucinogenic, from ergot alkaloids.
Mescaline	Hallucinogenic, from peyote cactus
Cannabis	Hallucinogenic,form the resin of cannabis sativa
Purine bases	Stimulate mental activity; coffee, tea, cocoa, kola, mate` etc.
Cocaine	Mental stimulant, from the leaves of *Erythroxylum coca.*
Ginkgo biloba	Improves short term memoty
Ginseng	Improves mental concentration particularly in the elderly.
Galanthamine	Contain alkaloidsfor treatment of Alzheimer's desease
Hypericum	Popular hrbal remedy for relief of mild moderate depression.
Sage	Revived interest in its use for counteracting memory loss.
Reserpine	Depresses mental activity, used in psychiatric treatment.
Yohimbine	Similar as reserpine, found in various species of the Apocynaceae
Valerian, passiflora	Sedative and hypnotic; aid sleeplessness and improve sleep quality
Analeptic drugs (stimulants of the CNS in addition to the mental stimulants indicated above).	
Picrotoxin	Treatment of barbiturate poisoning, found in *Anamirta cocculus*
Logeline	Obtained from *Lobelia inflata*
Strychnine	Weak analeptic; toxic doses produce spinal convulsions, from *strychnos spp.*
Camphor	Weak analeptic. Obtained from *Cinnamomum camphora.*
Central nervous system	
Tropane alkaloids	Effective in the alleviation of the symptoms of Parkinson's disease. Used in treatment of travel sickness and delirium tremens
Gelsemium root	Clinically to high toxicity. Occasionally used as antispasmodics
Analgesic drugs	
Morphine	Effective for relief of severe pain. The principal alkaloid of opium.
Codeine	Although less active than morphine it is a much safer drug for the relief of mild pain and for use as a cough suppressant

Drugs acting on the urinary and reproductive systems:

Diuretics	Xanthine derivatives as present in many beverages (tea, coffee.) promote dilation of the renal medullary blood vessels. Digitalis glycosides improve th failing heart thereby increasing renal perfusion and glomerular filtration;
Diuretics and urinary antiseptics	Buchu, beaberry, juniper, copaiba. These include drugs used for the treatment of cystitis and urethritis.
Drugs acting on the uterus.	Preparations of ergot were traditionally used in chilbirth and then largely replaced by the isolated alkaloid ergometrine. Administered as its salts it has a direct stimulant action on the uterine muscle and reduces the incidece of postpartum haemorrhage. Black haw is a uterine tonic and sedative used for the prevention of miscarriage and for dysmenorrhoea after childbirth. Hydrastis is employed for menorrhagia and other menstrual disorders.
Oral contraceptives	Numerous plants have been and are being, tested for antifertility activity.
Male impotence	Papaverine (under careful medical supervision), yohimbine(erectile dysfunction)
Benign prostate hyperplasia (BPH)	A number of phytoedicinals are imployed to treat the symploms of BPH.

Drugs used on the skin and mucous membranes:

Emollients and demulcents	These include a number of vehicles used in the preparation of ointments, creams, lotions, etc. and include fixed oils(e.g. olive, arachis, coconut, theobroma), fats (wool-fat, lard), waxes of animal origin (beeswax, spermaceti), gums (acacia, tragacanth) and mucilages (psyllium, elmback)
Absorbents	Starch, alginates, charcoal
Astringents	Tannis, krameria, catechu, galls, Aspidosperma, hamamelis, pomegranate rind, kinos.
Counter-irritants	Camphor, turpentine, capsicum, aconite, methyl salicylate, mustard seed.
Antiseptics	Tars, eucalyptus oil, thyme oil, eugenol, thymol, cajuput.
Anti-inflammatory agents	Corticosteroids used locally, matricaria, arnica.
Psoriasis and eczema treatment	Comfrey, allantoin, cadeoil, evening primerose oil, chrysarobin, lithospermum, savin, myrrh, grindelia.
Wound coverings	Type of wound covering is important in the healing process.

1.3 Description of family Dilleniaceae:

1.3.1 General description:

Trees or shrubs[38], sometimes climbing, or herbs; leaves alternate, simple, entire or toothed; but petiole sheathing, more rarely with lateral deciduous stipules. Flowers regular, hermaphrodite, often showy, white of yellow. Sepals 5, rarely more of fewer, imbricate, persistent, often accrescent. Petals 5, rarely more or fewer, caduceus. Stamens many, hypogenous; anthers innate; dehiscence longitudinal, introrse of lateral, or by terminal pores. Carpels 1-many, free or cohering; styles free, stigma simple; ovules soilitary amphitropous, of few ascending, or numerous on the ventral suture. Fruit indehiscent berry-like, or dehiscent follicular. Seeds 1 or few, arillate, rarely rather numerous and (Dillenia) exarillate; testa crustaceous, raphe short; albumen fleshy; embryo minute. This family consists of more than 400 species in 10 genous. It is widely found in tropical and subtropical region.

1.3.2 Medicinal importance of Dilleniaceae family:

The fruit juice of *D. indica* is used as expectorant in cough mixtures. The fruit of *D. indica* is said to possess tonic and laxative properties and used for abdominal pain. The leaves and bark are used as astringent. The alcoholic extract of the leaves of *D. indica* possessed CNS depressant activist in mice[39] (Bhakuri, et al, 1969). The seed of *D. indica* showed antimicrobial activity[40] (Lakshmi, et al., 1979).

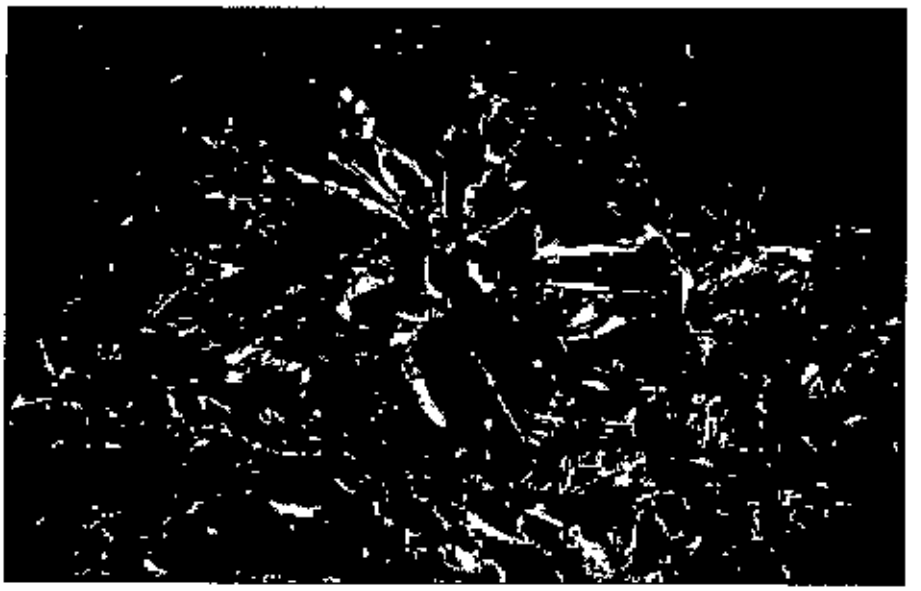
The *Dillenia pentagyna* Roxb (Beng: Banchalta, Hargeza, Fam: Dilleniaceae)is a medicinal plant and widely distributed throughout Bangladesh. The plant is said tto bew used in T.B., fistula, sore, carbuncle, neuralgia, pleur and pneum[41] (Asolkar, et al., 1992).

1.4 General description of the genus *Dillenia pentagyna*:

A large deciduous[38,42] tree with a straight cylindrical bole, 30-70 ft. In height and 8-10 ft. in girth, bearing a rounded crown of large leaves, 2-3ft. long nd 0.5-1ft. broad. Flowers are yellow, numerous fragrant, fruits orange yellow, 0.5-0.75 inch, in diam. Succulent and edible. *D. pentagyna* is available in Modhupur forest, Tangail. It is also found in Khagrachari district.

Picture-1: Photograph of *Dillenia pentagyna* tree.

Picture-2: Collecting the leaves of *Dillenia pentagyna*



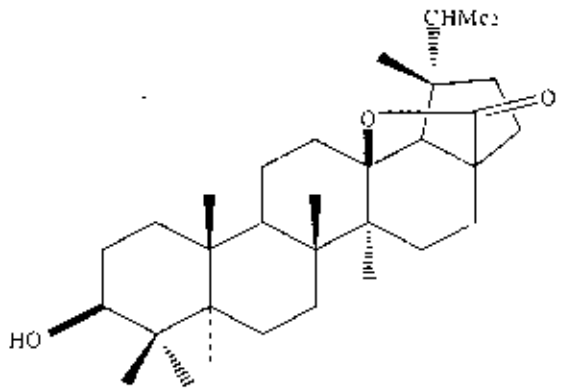
Picture-3 (a): Close photograph of *Dillenia pentagyna* leaves

Picture-3 (b): Close photograph of *Dillenia pentagyna* leaves

1.5 Chemical investigations of *Dillenia indica* and *Dillenia penagyna*:

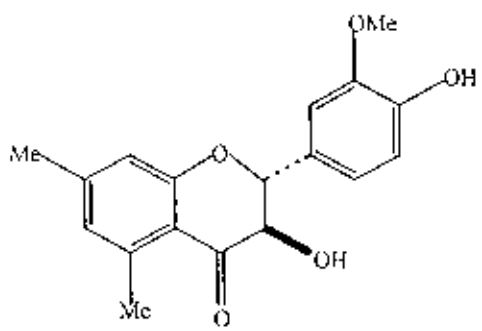
An extensive literature survey on the genus Dillenia showed that a lot of chemical work has been done on different species of this genus. These are as follows:

Nilima and her co-workers[43] in 1975 isolated a new pentacyclic triterpene lactone, 3β-hydroxy lupane-13β, 28-lactone (25) with betulinaldehyde, betulin, lupeol, β-sitosterol, berulinic acid and myricetine from the stem bark of *D. indica*.

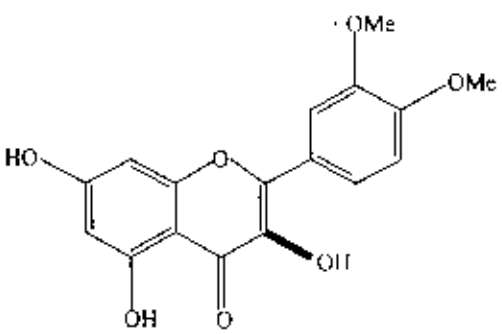


3β-hydroxy lupane-13β, 28-lactone (25)

Pavanasasivam[44], et al, in 1975 reported two flavone, 3.4',5,7 tetrahydroxy-3'-methoxy flavone (dihydroisorhametin, 26) and 3, 5, 7 trihydroxy-3'-4'-dimethoxy flavone (dillenetin, 27) from the *D indica*.



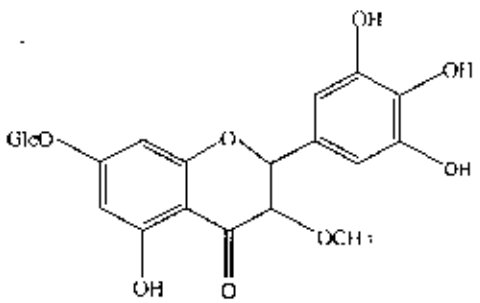
Dihydroisorhametin, (26)



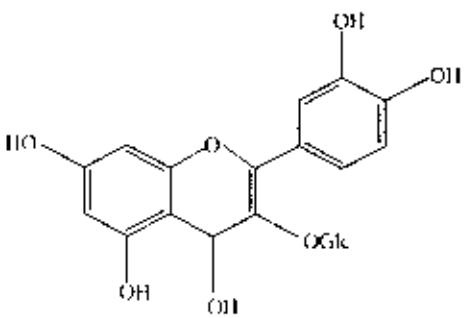
Dillenetin, (27)

In 1976, Sundararamaiah, et al[45].. reported the isolation of betulin and β-sitosterol from the heartwood of *D. indica*.

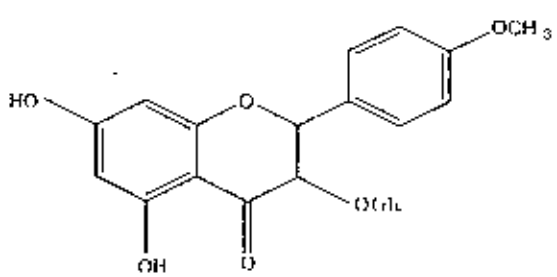
Tiwari, et al[46].. 1979 isolated and identified four compounds, 3`, 5`-dihydroxy, 4`, 3-dimethoxy flavone-7-*O*-β-D-glucopyranoside (28), 4,5,7,3`, 4` pentahydroxy flavon-3-*O*-β-D-glucopyranoside (29) and 5,7-dihydroxy, 4`-methoxy flavone-3-*O*-β-D-glucopyranoside(30) from the stem bark of *D. indica*



3`,5`-dihydroxy, 4`, 3-dimethoxy flavone-7-*O*-β-D-glucopyranoside (28)

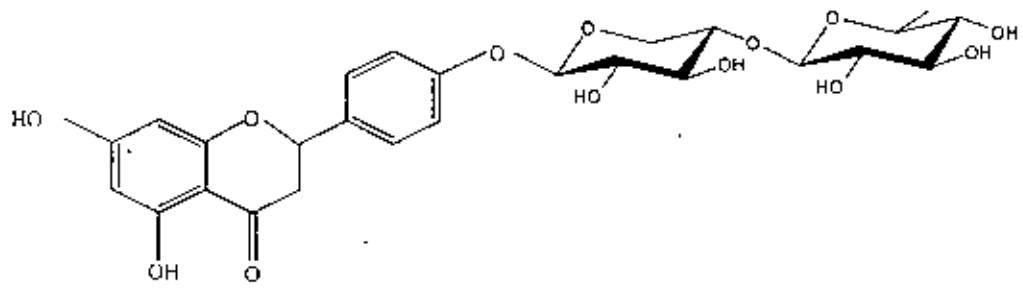


4,5,7,3`,4` pentahydroxy flavan-3-*O*-β-D-glucopyranoside (29)



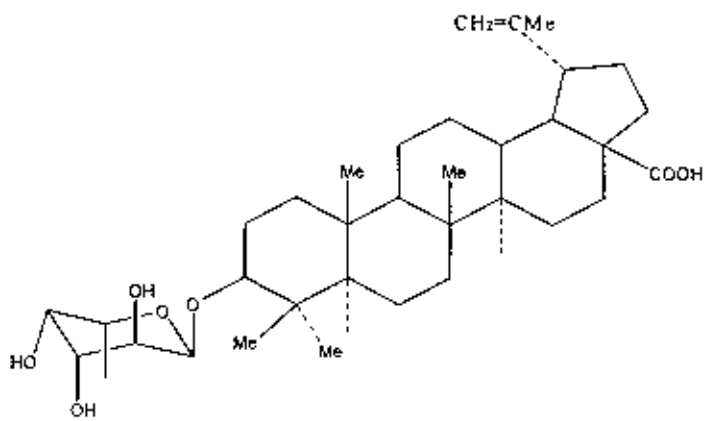
5,7-dihydroxy, 4`-methoxy Flavone-3-*O*-β-D-glucopyranoside (30)

In 1979, Tiwari et al[47].. reported a compound. Naringenin-4`-*O*-[4-*O*-(β-D-glucopyranosyl)-β-D-xylopyranoside] (31) from the stem bark of *D. pentagyna*.



Naringenin-4'-*O*-[4-*O*-(β-D-glucopyranosyl)-β-D-xylopyranoside] (31)

Kamala P. Tiwari[48], Savitri D. Srivastava and Santosh K. Srivastava reported the isolation and identification of a new saponin named α-L-rhamnopyranosyl-3β-hydroxy-lup-20 (29)-en-28-oic acid (32) on the basis of spectral and chemical evidence.

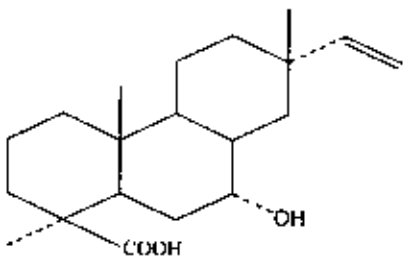


α-L-Rhamnopyranosyl-3-β-hydroxy-lup 20 (29)-en-28-oic acid (32)

Chief content of the fleshy sepals are tannin, malic acid, arabino-galactan and glucose. They also contain an arabinogalactan, betulin, betulinic acid. and flavonoids. Bark and wood contains flavonoids, betulin, betulinic acid. betulinaldehyde, lupeol, β-sitosterol, myricetinhydroxylactone, dihydroisorhamnelin, dilletin and glucosides. Leaves contain flavons, cycloartenone, betulinic acid, n-hentriacontanol and β-sitosterol.

The earlier work on the stem of the plant *Dillenia pentagyna* (N. O. Dilleniaceae) was reported by Tiwari et al[49]. In this paper the authors reported the isolation and

characterization of a new diterpene, dipoloic acid [7-hydroxy-pimara- (15:16)-ene-19-oic acid], (33) by physico-chemical techniques.



[7-hydroxy-pimara- (15:16)-ene-19-oic acid, (33)]

1.6 Objective of the present research work:

Medicinal plants are selected from published folkloric use of plants, information from ethno botany and ethno pharmacological reports. Plant materials are also selected from chemotaxonomic data. Sometimes medicinal plants are also studied by selecting medicinal species of the active genera.

A collaborative research program was done by National Cancer Institute (NCI) Maryland, USA and Department of Chemistry University of Dhaka on the Biological activity of some of the Bangladeshi plant material; *Dillenia pentagyna* was one of them

From the literature survey it is evident that a number of compounds have been isolated from the bark of *Dillenia pentagyna* but the leaves were not studied in detail. Therefore, the present program is designed to study the leaves of *Dillenia pentagyna*. In this program chemical studies will be carried out to isolate pure compounds from the plant extracts by various chromatographic methods. Then the isolated compounds will be characterized by chemical and spectroscopic methods.

CHAPTER 2

2.0 EXPERIMENTAL

2.1 General methods:

The following sections of this chapter are a brief description of the various method followed in extraction, fractionation and purification of the compounds in the course of experimental works.

2.1.1 Solvents and Chemicals:

Analytical or laboratory grade solvents and chemicals were used in all experiment and these were procured from E. Merck (Germany) and BDH (England). Commercial grade of dichloromethane, ethyl acetate methanol, acetone and absolute alcohol were distilled prior to use for extraction and chromatographic separation.

2.1.2 Evaporation:

All evaporation was performed at reduced pressure using rotary vacuum evaporator at bath temperature not exceeding 40^0C. Small volumes of non-aqueous solvents were concentrated by blowing with nitrogen at room temperature.

2.1.3 Freeze-drying:

All freeze-drying operations were carried out with a Varian 801 model LY-3-TT and HETOSICC (Denmark) freeze dryer. Organic solvents were completely removed by evaporation and using drying pumps before freeze-drying. Aqueous extracts and fractions were first frozen in round-bottomed flasks in a methanol freezer (HETOFRIG CB5, Denmark) at -30^0C to -35^0C and finally the materials were subjected to freeze during operation.

2.1.4 Chromatographic techniques:

2.1.4.1 Thin layer chromatography (TLC):

Two types of TLC plates were used throughout the experiments:

(i) Pre-coated TLC plates: 0.2 mm thin coating of silica gel GF_{254} on aluminium sheets and

(ii) Manually prepared glass plates.

(a) Preparation of the plates:

Thin layer chromatographic (TLC) plates were prepared by spreading a film of aqueous slurry (gel: water=1:2 w/v) of silica gel G_{60}, GF_{254} (E Merck, 7731) over the entire surface of the 2x8 cm glass plates in 0.2 m thickness. The plates were dried in air and finally activated by heating at 110^0C for 1 hour followed by cooling at room temperature.

(b) Application of the sample and development of the plates:

For application of the samples capillary tubes were used The TLC plates were developed by the ascending technique in TLC tank using selected solvent system

(c) Solvents system:

The solvents of different polarity used for thin layer chromatography are given below:

(1) For less polar fractions and compounds binary solvent systems were used e.g.,

(i) Dichloromethane: ethyl acetate (in different ratio)

(ii) Dichloromethane: methanol (in different ratio)

(iii) Ethyl acetate: methanol (in different ratio)

(2) For more polar fractions and compounds trinary solvent systems were used. e.g.,

(i) Dichloromethane: methanol: water (in different ratio)

(d) Locations of spots:

Irrigated plates were developed by one of the following methods to detect the position of the spots.

(i) **UV light:** Examination under an UV light source with two different wave lengths (254 nm and 350 nm)

(ii) **Iodine vapour:** The plates were exposed to iodine vapor for a few minutes to detect the location of spots.

(iii) **Vanillin in sulfuric acid (1%):** The plates were sprayed with 1% vanillin in concentrated sulfuric acid and then heated for 10 minutes at 120^0C to visualize the spots of bands.

(iv) **Ceric (IV) sulfate in sulfuric acid (1%):** The plates were sprayed with the reagent followed by heating in an oven at 120^0C for 10 minutes.

2.1.4.2 Column chromatography:

(a) Column:

Glass columns of different sizes glass tube (90cmx10cm,i.d. and 60cmx3cm,i.d) fitted with a rota flow and small burette like glass column (30cm x 1cm i.d.) fitted with Teflon flow control unit were used for chromatographic separations.

(b) Stationary phase:

For normal phase chromatography column grade silica gel 60-230 was used. For reverse phase chromatography Sephadex LH-20 gel (particle size 25-100mm, Pharmacia, Sweden).

(c) Preparation of silica gel flash column:

To prepare particular column, the required amount of silica gel was swelled into a selected solvent (e.g. hexane, dichloromethane, ethyl acetate or a mixture of different solvents in different ratio) and then poured into the column with continuous flow of the solvent. For homogeneous packing, the column was equilibrated with two to three column volumes of solvent. Normal phase column chromatographic separation was performed by gravitational flow with solvents of increasing polarity. A fish aquarium air pump (EK 8000) was used to apply pressure in the flash column. The air pressure was provided with a vain tube. The airflow from the aquarium pump was adjusted to give the required pressure (1-2 bar) for a suitable elution speed.

(d) Preparation of Sephadex LH 20 gel column:

The required amount of sephadex LH 20 gel (particle size 25-100mm, Pharmacia, Sweden) was suspended in water containing 0.1% 1-butanol (preservative) for two hours .The gel was degassed (~30 min) with occasional shaking .The column was conditioned with three column volumes of water followed by methanol and acetone. The column was then equilibrated with three column volumes of water. The conditioned column was then ready for sample application.

(e) Application of the sample into the column:

The crude extract or sub fraction thereof was applied into the column either as a solution or in a powdered form. To apply the extract in powder form the sample was dissolved in a particular solvent or a mixture of solvents and silica gel (sample: gel, 1:2 w/w) was added to the sample solution and the mixture was evaporated to dryness. The dried materials were ground thoroughly in a mortar to make fine powder and the powder was then applied into the column.

To apply the sample in the form of solution it was dissolved in a minimum volume of the column equilibrating solvent and was applied into the column. The sample layer was levelled by gentle tapping on the column. On top of this layer about 0.5-1 cm of the silica gel was placed so that the surface of the bed was not affected during solvent application.

(f) Fractionation and monitoring procedure:

After application of the sample, the columns were eluted with the equilibrating solvent and the polarity of the mobile phase was either increased gradually by adding hexane, dichloromethane, ethyl acetate and methanol (for silica gel column) or decreased gradually by adding water, methanol and dichloromethane (for Sephadex LH-20). The eluted samples were collected in conical flasks or test tubes and the fractions were monitored by TLC using different solvent systems (sec 2.1.4.1, C). Fractions were combined on the basis of their R_f^{50} values.

2.1.5 Spectroscopic methods:

(a) Ultraviolet (UV) spectroscopy:

A Shimadzu UV 160 A recording spectrophotometer was used to record Ultraviolet (UV) spectra. The sample was dissolved in a suitable solvent and the solution was taken in a 1cm x 1cm quartz cell for recording the spectrum. Main bands (λ_{max}) were recorded as wave length (nm).

(b) Infrared (IR) spectroscopy:

The Infrared (IR) spectra were recorded as KBr pellets using a Shimadzu FT-IR-8101 spectrophotometer. Major bands were recorded in wave number (cm^{-1}).

(c) Nuclear magnetic resonance (NMR) spectroscopy:

The ^{1}H and ^{13}C-NMR spectra of pure compounds were recorded on a Brukker 400 MHz spectrometer using $CDCl_3$ (for non Polar compounds) and CD_3OD (for polar compounds). Tetra methyl silane (TMS) was used as internal reference in every case. The DEPT experiments of ^{13}C-NMR spectra were obtained by varying the pulse width (0) by 135°. The chemical shift (δ) values were reported in ppm and coupling constants (*J*) were measured in Hz.

2.1.6 Melting point:

Melting points were rerecorded by inserting a small amount of the sample into a capillary tube and then measured in an OGAWA SEIKI melting point apparatus.

2.1.7 Preparation of vanillin-sulfuric acid reagent:

To prepare vanillin sulfuric acid reagent, one gram of vanillin was dissolved in 100 ml of concentrated sulfuric acid. The prepared solution was then kept in a glass made sprayer. Irrigated TLC plates were sprayed and heated at 120°C for 10 minutes.

2.1.8 Preparation of Ceric (iv) sulfate-sulfuric acid reagent:

One gram of Ceric (iv) sulphate was dissolved in 100 ml of 5N sulfuric acid to prepare this reagent. The prepared solution was kept in a glass made sprayer.

2.1.9 The Salkowski test for steroids:

Small amount of sample was dissolved in a mixture of chloroform and methanol, then a few drops of concentrated sulfuric acid was added to the solution. A reddish color developed for steroids[31].

2.2 Chemical studies

2.2.1 Collection of plant materials:

Leaves of *Dillenia Pentagyna* were collected from Modhupur forest, of the district Tangail, Bangladesh. The leaves were collected from matured tree with the help of a taxonomist of Bangladesh National Herbarium (BNH). A voucher specimen was made and it was submitted to the BNH (Bangladesh National Herbarium) for the reference (DACB Accession No. 29447). The leaves were cut into small pieces, air-dried and finally dried in an oven at 40°C. The dried leaves were made into powder by grinding in a Cyclotec grinding machine (200 mesh). The powder was stored in airtight wide-mouth bottles and was used for subsequent extractions.

2.2.2 Extraction

(a) Extraction of plant powder with dichloromethane: methanol:

The dried and powdered leaves of *Dillenia Pentagyna* (1.9 kg) was taken in a few precleaned cloth bags. The bags were transferred into a stainless steel tank. Mixture of DCM:MeOH (1:1) was added into the tank so that the solvent immersed the whole bag. The extract was collected after 24 hours, filtered by Buckner funnel and concentrated by a rotavapor. Similar percolations were carried out for three more times. All the concentrated extracts were combined, evaporated to dryness and finally dried by high vacuum in a freeze-dryer to give 69.39g (3.65 %) of solid material (Scheme-1).

(b) Partition of DCM: MeOH extract:

DCM: MeOH extract (69.39g) of leaves was suspended in water (0.5L) and the suspension was transferred into a separating funnel. The aqueous suspension was partitioned with dichloromethane (1Lx3) at room temperature using a separating funnel. After partition two parts were obtained aqueous and dichloromethane part. The dichloromethane part was evaporated to dryness (33.68g). The aqueous suspension was treated with ethyl acetate (0.5L x 3). The ethyl acetate soluble layer was separated and evaporated to dryness (2.12g). The remaining aqueous part was further partitioned with 1-butanol (0.75Lx3). The 1-butanol soluble part was separated and evaporated to dryness (3.5g). The DCM extract was further partitioned (Scheme-2) with aq. 90% MeOH :

1-butanol (0.75Lx3). The 1-butanol soluble part was separated and evaporated to dryness (3.5g). The DCM extract was further partitioned (Scheme-2) with aq. 90% MeOH : Hexane (1:1). Two parts were obtained, Hexane and aq. 90% MeOH part. Both the parts were evaporated to dryness and the solid mass was 10.05g and 19.16g respectively. Percentage of different parts of DCM:MeOH (1 : 1) extract of *Dillenia pentagyna* is given in table-1.

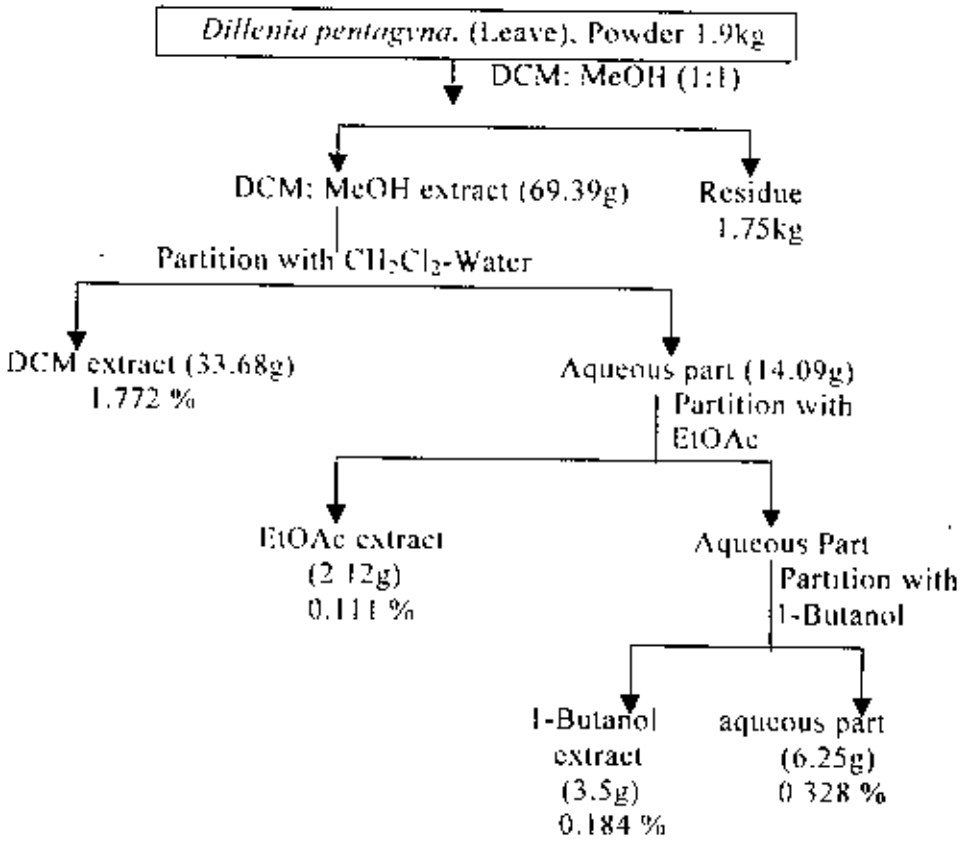
Table-1: Percentage of different parts of DCM: MeOH (1: 1) extract of *Dillenia pentagyna* leaves.

Parts	Amount (g)	% of dry parts
DCM extract	33.68	1.772
EtOAc	2.12	0.111
1-Butanol	3.5	0.184
Aqueous part	6.25	0.328

Table-2: Percentage of different parts of DCM extract of *Dillenia pentagyna* leaves.

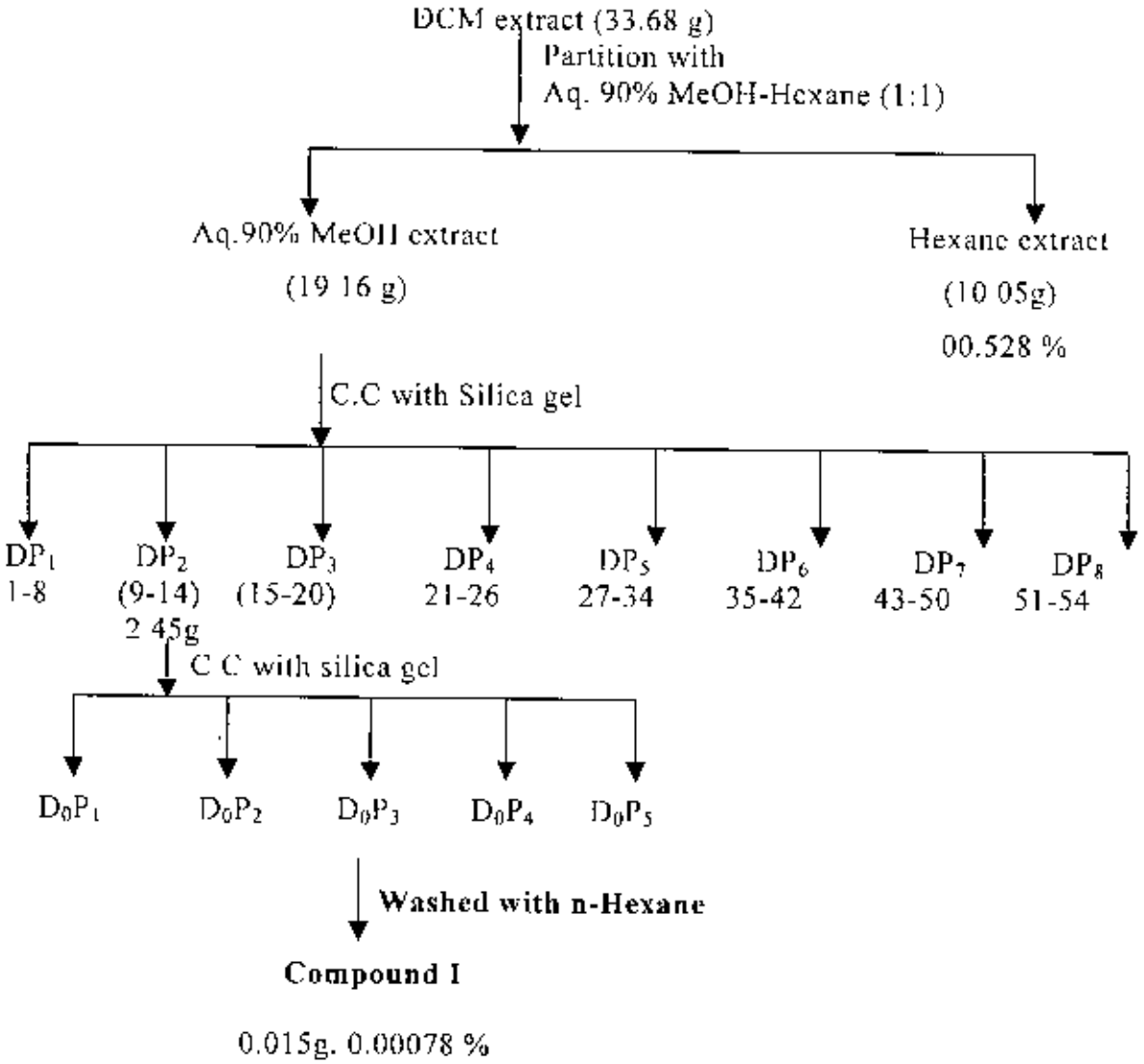
Parts	Amount (g)	% of dry parts
Aqueous 90% MeOH extract	19.16	1.008
Hexane	10.05	00.528

Extraction of *Dillenia pentagyna*



Scheme-1

Column chromatography of DCM extract



Scheme-2

2.2.3 Fractionation of dichloromethane extract by silica gel column:

(a) Preparation of silica gel column:

A glass column (80 cm x 20 cm: i. d.) was packed with 250g of column grade silica gel using hexane as equilibrating solvent. The column was flashed with three column volumes of dichloromethane and finally the column bed became 26 cm in length.

(b) Preparation of sample and application:

Aqueous 90% MeOH soluble part (19.16g) was dissolved in dichloromethane and methanol mixture and silica gel (50g) was added to the solution. The slurry was evaporated to dryness by vacuum evaporator. This was further dried with freeze-drier and made into fine powder by mortar and pestle.

(c) Fractionation of the applied sample:

The dried sample was applied on the top of the column bed. The applied sample was first eluted by dichloromethane and the polarity of the solvents was gradually increased by adding methanol. The eluted samples were collected in 54 conical flasks and monitored by TLC and were combined on the basis of their R_f values and finally eight fractions (DP_1-DP_8) were obtained. The amount of each fraction and their TLC patterns are given in Table-3.

Table-3: Amount of each fraction and their TLC patterns of DCM extract.

Fraction No.	Amount (g)	TLC pattern
DP_1	0.85	Spot with long tailing
DP_2	2.45	Spot with very small tailing
DP_3	2.68	Tailing
DP_4	1.13	Tailing
DP_5	1.45	Tailing
DP_6	1.65	Tailing
DP_7	0.95	Tailing
DP_8	1.20	Tailing

2.2.4 Fractionation of DP_2:

(a) Preparation of Silica gel column:

A glass column was packed with column (60-230) grade silica gel (48g) using hexane as the eluting solvent. The column was then equilibrated with three column volumes of dichloromethane.

(b) Preparation of sample and application:

Fraction DP_2 (2.45g) was dissolved in dichloromethane and methanol mixture and silica gel (5g) was added to the solution. The slurry was evaporated to dryness by vacuum evaporator. This was further dried with freeze-drier and made into fine powder by mortar and pestle.

(c) Fractionation of the applied sample:

The dried sample was applied on the top of the column bed. The applied sample was first eluted by n-Hexane and the polarity of the solvents was gradually increased by adding dichloromethane. The eluted samples were collected in 30 conical flasks and monitored by TLC and were combined on the basis of their R_f values and finally five fractions (D_0P_1-D_0P_5) were obtained. The amount of each fraction and their TLC patterns are given in the table-4.

Table-4: Amount of each fraction and their TLC patterns DP_2.

Fraction No.	Amount (g)	TLC pattern
D_0P_1	0.500	Spot with long tailing
D_0P_2	0.150	Spot with small tailing
D_0P_3	0.015	Round spot
D_0P_4	0.130	Spot with tailing
D_0P_5	0.350	Tailing

2.2.5 Isolation of compound I from D_0P_3:

The fraction D_0P_3 (0.015g) was orange colored material. It was washed with hexane for several times to remove associated coloring materials. After washing a white solid material was obtained (0.015g) which was dissolved in CH_2Cl_2. The TLC of the compound showed a single spot in dichloromethane: hexane (1:1).

2.2.6 Properties of the compound I:

(a) Physical properties:

The compound I (0.015g) was white crystalline compound. The R_f value of the compound was 0.45 with 50% dichloromethane in hexane. It had a melting point of 128-129^0 C. It was soluble in dichloromethane. It was tested by Salkawoski method, which developed a reddish color indicating that the compound may be a steroid.

(b) Characterization of the compound I by spectroscopic methods:

(i) Ultraviolet (UV) spectroscopy:

The UV spectrum (Fig-1) of the compound I had absorption at λ_{max} 280nm and 229nm (MeOH).

(ii) Infrared (IR) spectroscopy:

The IR spectrum (in KBr) (Fig-2) of the compound-I had important absorption frequencies at (KBr) 3421.5 (OH), 2937.4 (-CH_3) and 2889.2 (-CH_3) cm^{-1}.

(iii) ^{1}H-NMR spectroscopy:

The ^{1}H-NMR spectrum (400 MHz, $CDCl_3$) (Fig-3) of the **compound-I** had signals at δ_H (ppm) 5.351 (1H, s, H-6), 5.151 (1H, m, H-23), 5.031 (1H, m, H-22), 3.523 (broad singlet, oxymethine proton, H-3), 1.442-2.283 (due to methylene and methine protons), 0.687 (3H, d, *J*=6), 0.804 (3H, S), 0.834 (3H, d, *J*=8.4), 0.921 (3H, d, *J*=5.6), 1.009 (3H, S), 1.109 (3H, S), 1.253 (3H, S) (7 methyl protons).

(iv) ^{13}C-NMR spectroscopy:

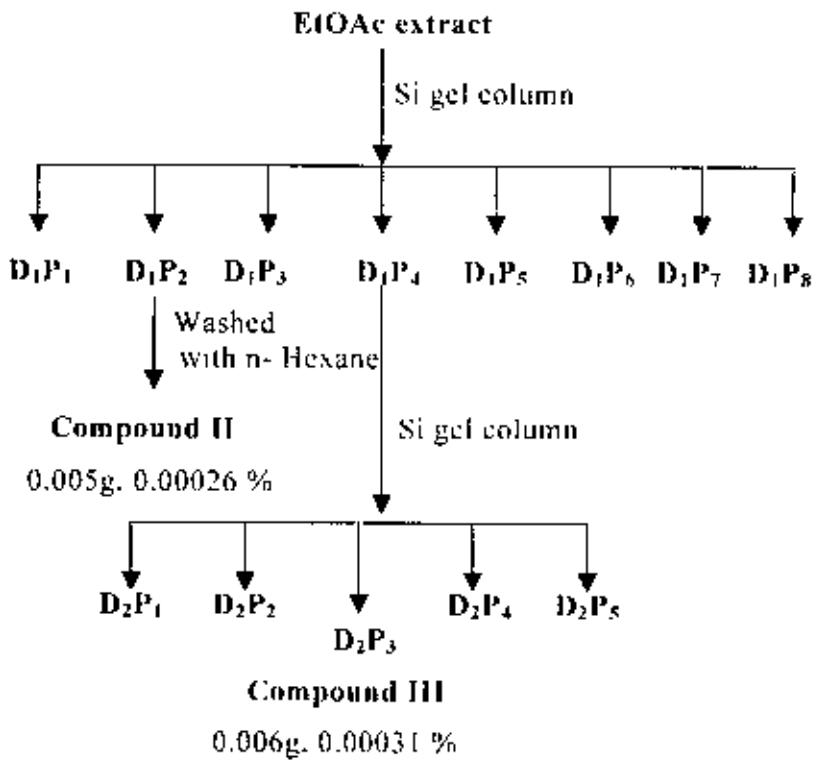
The ^{13}C-NMR spectrum ($CDCl_3$) (Fig-6) of the compound I had signals at δ_C 37.291, 31.695, 71.819, 42.339, 140.88, 121.724, 31.902, 31.943, 50.169, 36.17, 21.117, 39.809, 42.425, 56.798, 24.389, 28.273, 56.095, 11.885, 19.420, 36.173, 18.809, 33.980, 26.122, 45.87, 29.193, 19.841, 19.068, 23.101, 12.269, 39.714, 42.339, 56.896, 28.936, 55.991, 12.009, 40.510, 138.33, 129.309, 51.265, 21.242, 19.012, 25.427 and 12.073 ppm

2.3 Fractionation of the EtOAc extract:

(a) Preparation of Silica gel column:

A glass column was packed with column grade silica gel (160g) using a mixture of dichloromethane and hexane (1 1) as eluting solvent. The column was then equilibrated with three column volumes of dichloromethane.

Column chromatography of EtOAc extract



Scheme - 3

(b) Preparation of the sample and application:

The ethyl acetate extract (2.0g) was dissolved in sufficient mixture of dichloromethane methanol (1:1) and treated with charcoal to get rid of the associated chlorophyll. After the charcoal treatment it was absorbed on silica gel 4.0g and the slurry was evaporated to dryness by a rotavapour followed by high vacuum pump. This was further dried with a freeze-drier and made into fine powder by mortar and pestle.

(c) Application and fractionation of the sample:

The dried sample was applied on the top of the column bed. The applied sample was eluted using dichloromethane followed by methanol. The polarity of the solvent was gradually increased. The eluted samples were collected in 48 conical flasks (25ml) and monitored by TLC and were combined on the basis of their R_f values and finally eight fractions (D_1P_1-D_1P_8) were obtained. The amount of each fraction and their TLC pattern are given in Table-5

Table-5: The amount of different fractions obtained by column chromatography:

Fraction	Amount (g)	TLC pattern
D_1P_1	0.15	Tailing
D_1P_2	0.05	Round spot with very small tailing
D_1P_3	0.20	Tailing
D_1P_4	0.30	Round spot
D_1P_5	0.09	Tailing
D_1P_6	0.15	Tailing
D_1P_7	0.14	Tailing
D_1P_8	0.11	Tailing

2.3.1 Isolation of compound II from D_1P_2:

Fraction D_1P_2 gave round spot. It was further washed with n-hexane for several times to remove associated coloring materials. The residue was crystallized with EtOAc-MeOH mixture. The crystals were dried and compound II 0.005g was obtained.

2.3.2 Characterization of compound II:

(a) Physical properties

The compound II was obtained as colourless needles and were soluble in a mixture of chloroform and methanol. Melting point of the sample was found to be 278-280°C. It gave violet colour with vanillin-sulphuric acid reagent indicating that compound II might be a triterpene.

(b) Characterization of the compound II by spectroscopic methods:

(i) Ultraviolet (UV) spectroscopy:

The UV spectrum (Fig: 13) of the compound was recorded and was found that the compound had absorption maximum at λ_{max} 229.0 nm and 281.0 nm in methanol.

(ii) Infrared (IR) spectroscopy:

The IR (KBr pellet) spectrum (Fig: 14) of the compound II had major absorbances at 3459.8 (-OH), 3000-2500 (-COOH), 2972.2 ($-CH_3$), 1685.7 (>C=O), 1451.3, 1376.1 and 882.4 (C-H) cm^{-1}.

(iii) ^{1}HNMR spectroscopy:

The ^{1}H-NMR (Fig: 15) of the compound-II had signals at 4.532 (1H, S, H-29), 4.661 (1H, S, H-29), 3.109 (1H, H-3, distorted doublet doublet), 2.89 (1H, H-19), 0.69 (3H, S, H-25), 0.76 (3H, S, H-27), 0.88 (3H, S, H-26), 0.89 (3H, S, H-23), 0.90 (3H, S, H-24), 1.62 (3H, S, H-30) ppm for six methyl groups.

(iv) ^{13}CNMR spectroscopy:

The ^{13}C-NMR spectrum (Fig. 18) of Compound II gave 30 signals at 38.69, 27.05, 78.85, 38.77, 55.32, 18.23, 34.29, 40.64, 50.51, 37.13, 20.84, 25.49, 38.26, 42.40, 30.55, 32.21, 56.17, 46.92, 49.18, 150.68, 29.62, 37.07, 27.84, 15.27, 15.84, 16.02, 14.59, 179.01, 109.42 and 19.24 ppm.

2.3.3 Fractionation of the D_1P_4:

(a) Preparation of Silica gel column:

A flash column (40cm x 2cm; i.d.) was packed with column (60-230) grade silica gel using EtOAc as the equilibrating solvent. The column was then equilibrated with three column volumes of EtOAc.

(b) Preparation of the sample and application:

The sample (0.30g) was dissolved in EtOAc and column (60-230) grade silica gel 0.40g was added to it and evaporated to dryness. It was further dried with a freeze-dryer and made into fine powder by mortar and pestle. The dried sample was applied to the column.

(c) Fractionation of the applied sample:

The sample was then eluted by ethyl acetate and the polarity of the solvent was gradually increased by the addition of methanol. The eluted samples were collected in different conical flasks and five different fractions were obtained according to their R_f values on TLC plates. Amount of each fraction and their TLC patterns are given in table-6

Table-6: different fractions of EtOAc part obtained by column.

Fraction	Amoumt (g)	TLC pattern
D_2P_1	0.009	Tailing
D_2P_2	0.01	Tailing
D_2P_3	0.006	Round spot
D_2P_4	0.02	Tailing
D_2P_5	0.025	Tailing

2.3.4 Isolation of compound III from D_2P_3:

Fraction D_2P_3 gave round spot. It was further washed with n-hexane for several times to remove associated coloring materials. The residue was crystallized with EtOAc-MeOH mixture. The crystals were dried and compound III (0.006g) was obtained

2.3.5 Properties of the compound III:

(a) Physical properties

The compound III was soluble in a mixture of chloroform and methanol. Melting point of the sample was found to be 277-279°C. It gave violet color with vanillin-sulphuric acid reagent indicating that compound III might be a triterpene.

(b) Characterization of the compound III by spectroscopic methods:

(i) Ultraviolet (UV) spectroscopy:

The UV spectrum (Fig: 23) of the compound was recorded and was found that the compound had absorption maximum at λ_{max} 228.0 nm in methanol.

(ii) Infrared (IR) spectroscopy:

The IR (KBr pellet) spectrum (Fig: 24) of the compound III had major absorbance at 2939.3 cm^{-1} for -CH_3 str and 1735.8 cm^{-1} for ester C=O str

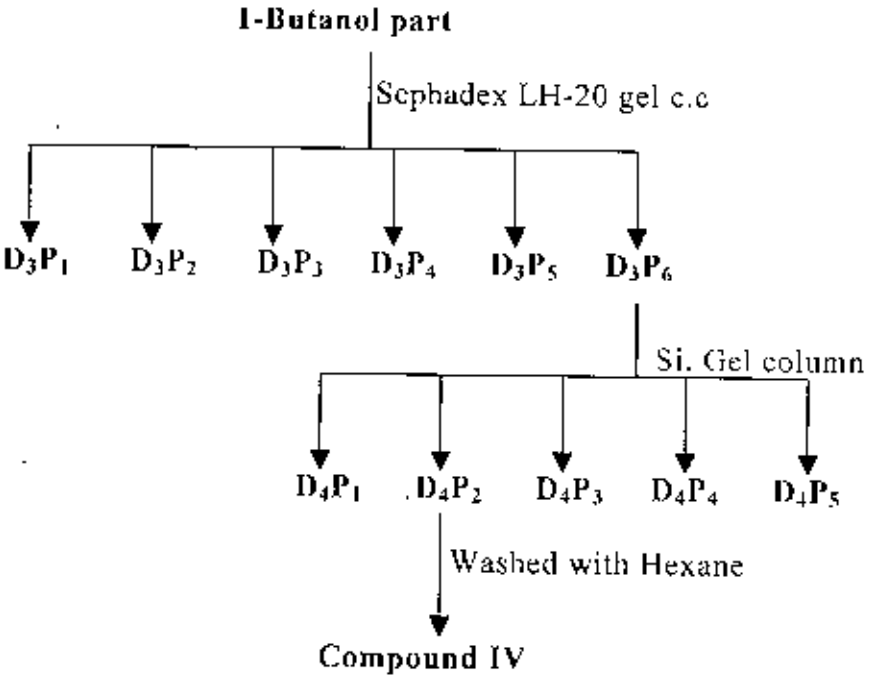
(iii) ^{1}H-NMR spectroscopy:

The ^{1}H-NMR (Fig: 25) of the compound-III had signals at 4.44 (1H, m, H-3) 4.674 (1H, S, H-29a), 4.558 (1H, s, H-29b), 1.674 (3H, s, H-30), 0.778 (3H, S, H-23), 0.846 (3H, S, H-24), 0.836 (3H, S, H-25), 0.93 (3H, S, H-26), 1.022 (3H, s, H-27), 1.38 (3H, s, H-28) and 2.02 (H-32, S)

(iv) ^{13}C-NMR spectroscopy:

The ^{13}C-NMR spectrum (Fig: 27) of Compound III gave 32 carbon signals at 38.454, 23.762, 81.026, 38.102, 55.451, 18.257, 34.281, 40.90, 50.403, 37.134, 21.003, 25.174, 37.837, 42.874, 27.493, 35.627, 43.030, 48.350, 48.041, 150.974, 29.900, 40.046, 27.979, 16.015, 16.200, 16.513, 14.541, 18.032, 109.378, 19 332, 171.010 and 21.327ppm

Column chromatography of 1-Butanol extract



β-Sitosterol glucoside

0.0045g, 0.00023 %

Scheme-4

2.4 Fractionation of the 1-Butanol extract:

(a) Preparation of Sephadex LH-20 gel column:

Sephadex LH-20 gel (particle size 25-100mm. Pharmacia, Sweden) was suspended in 250ml of water. The gel was degassed (30 min) with occasional shaking .The column was conditioned with three column volumes of water followed by methanol and acetone. The column was then equilibrated with three column volumes of water. The conditioned column was then ready for sample application.

(b) Preparation of the sample and application:

The 1-Butanol extract (3.5g) was dissolved in a minimum volume of water. It was then centrifuged and the residue was discarded. The centrifuge was kept in refrigerator for 24 hrs. Then it was centrifuged again. After discarding the residue the clear centrifuge was concentrated and applied to the column.

c) Fractionation of the sample:

The column was eluted with H_2O and with mixture of solvents water and methanol. The eluted fractions were collected in different conical flasks According to the TLC pattern six fractions (D_3P_1-D_3P_6) were obtained. Each fraction were evaporated to dryness and finally dried by high vacuum in a freeze-drier.

Table-7: Different fractions of the 1-Butanol extract obtained by column chromatography:

Fraction	Amoumt (g)	TLC pattern
D_3P_1	0.156	Tailing
D_3P_2	0.200	Tailing
D_3P_3	0.100	Tailing
D_3P_4	0.090	Tailing
D_3P_5	0.100	Tailing
D_3P_6	0.110	Spot with tailing

2.4.1 Fractionation of D_3P_6:

(a) Preparation of Silica gel column:

A flash of column (40cm x 2cm; i.d.) was packed with column grade silica gel using dichloromethane as the equilibrating solvent. The column was then equilibrated with three column volumes of dichloromethane.

(b) Preparation of the sample and application:

The sample 0.110g was dissolved in dichloromethane and column grade silica gel 0.250g was added to it and evaporated to dryness. It was further dried with a freeze-dryer and made into fine powder by mortar and pestle. The dried sample was applied to the column.

(c) Fractionation of the applied sample:

The sample was then eluted by dichloromethane and the polarity of the solvent was gradually increased by the addition of methanol. The eluted samples were collected in different conical flasks and five different fractions were obtained according to their R_f values on TLC plates. Amount of each fraction and their TLC patterns are given in table-8.

Table-8: different fractions of D_3P_6 part obtained by column chromatography

Fraction	Amoumt (g)	TLC pattern
D_4P_1	0.010	Tailing
D_4P_2	0.0045	Round spot
D_4P_3	0.009	Tailing
D_4P_4	0.010	Tailing
D_4P_5	0.015	Tailing

2.4.2 Isolation of compound IV from D_4P_2:

Fraction D_4P_2 gave round spot. It was further washed with n-hexane for several times to remove associated coloring materials. The residue was white solid crystal. The crystals were dried and compound IV was obtained (0.0045g).

2.4.3 Properties of the compound IV:

(a) Physical properties

The compound IV was soluble in a mixture of dichloromethane and methanol. It was tested by Salkawoski method, which developed a reddish color indicating that the compound may be a steroid. Again a small amount of the compound was taken with 80% phenol and concentrated sulphuric acid was added with it, a reddish brown color developed indicating the compound may contain a sugar residue. Melting point of the sample was found to be 178^{0}C.

(b) Characterization of the compound IV by spectroscopic methods:

(i) Ultraviolet (UV) spectroscopy:

The UV spectrum (Fig: 31) of the compound was recorded and was found that the compound had absorption maximum at λ_{max} 229 nm in methanol.

(ii) Infrared (IR) spectroscopy:

The IR (KBr pellet) spectrum (Fig: 32) of the compound IV had major absorbance at 3565.1 for –OH, 2931.6 cm^{-1} for -CH$_3$ str, 1652.9 cm^{-1} for C=C str.

(iii) ^{1}H-NMR spectroscopy:

The ^{1}H-NMR (Fig: 33) of the compound-IV had signals at 3.20 (1H, m, H-3), 3.34-3.80 for oxymethine protons of glucose, 5.30 (1H, distorted doublet, H-6), 0.635 (3H, d, *J*=7.2, H-26), 0.752 (3H, s, H-27), 0.791 (3H, d, *J*=8, H-29), 0.868 (3H, d, *J*=6.4, H-21), 0.964 (3H, d, *J*=8.4, H-18), 1.201 (3H, s, H-19) and 4.35 (1H, d, *J*=7.6, H-1 of glucose) ppm.

(iv) ^{13}C-NMR spectroscopy:

The ^{13}C-NMR spectrum of Compound IV gave 35 signals at 36.544, 29.36, 78.795, 39.34, 121.764, 140.014, 33.761, 31.573, 49.893, 37.192, 20.725, 38.651, 42.521, 56.441, 25.549, 29.245, 55.990, 11.501, 19.334, 36.945, 18.894, 39.652, 22.721, 45.554, 18.561, 28.827, 19.334, 24.201, 11.359, 100.809, 73.251, 76.171, 69.904, 75.556 and 61.482 ppm.

CHAPTER 3

3.0 RESULTS & DISCUSSION

3.1 General:

The various sections of this chapter are a brief discussion of the work done on *Dillenia pentagyna* belonging to the family *Dilleniaceae*.

3.1.1 Plant material:

Dillenia pentagyna is one of the important medicinal plants of *Dilleniaceae* family. Fresh leaves of *Dillenia pentagyna* were collected (Sec 3.1.1) Leaves were cut into small pieces, air -dried, dried in an oven at 40^0C and powdered for the present work

3.2 Extraction of plant powder:

The powdered materials (1.9 kg) were extracted with DCM: MeOH (1:1). The extract was evaporated to dryness by a rotavapor and finally dried by high vacuum in a freeze –drier to give 69.39g of extract (Sec 3.1.2.a).

3.3 Partition of the extract:

The dried DCM.MeOH extract (69.39 g) was divided into four parts by partitioning with water, dichloromethane, ethyl acetate and 1-butanol

3.4 Further partition of the dichloromethane extract:

Dichloromethane part (33.68 g) was partitioned into two different fractions by using Aq. 90% MeOH - Hexane (1: 1) (Sec 3.1.2.b)

3.5 Investigation of Aq. 90% MeOH extract:

The Aq. 90% MeOH extract (14.09g) was fractionated to eight different fractions (DP_1 to DP_8, Table-3) by using dichloromethane and methanol as the eluting solvent (Sec 3.1.3 c). Fraction DP_2 (2.45g) was further fractionated by cc using silica gel. The eluted samples were collected in 30 conical flasks and monitored by TLC and were combined on the basis of their R_f values and finally five fractions (D_0P_1-D_0P_5) were obtained. D_0P_3 gave a round spot. It was washed with hexane for several times to remove associated coloring materials. After washing a white crystalline material was obtained which was dissolved in dichloromethane. The solution tested again by TLC and after recrystallization it was stored in a cool place.

3.6 Characterization of compound-I as the mixture of steroids:

3.6.1 Physical characteristics:

The compound I was white crystalline compound. It had a melting point 128-129°C. It was readily soluble in dichloromethane. It gave reddish color in the chloroform layer when tested by Salkawoski method and pink color with vanillin-sulphuric acid reagent. Which indicates the compound as a steroid. Therefore, it was considered that the compound might be a steroid.

3.6.2 Structure elucidation of the compound-I by spectroscopic methods:

(i) Ultraviolet spectroscopy:

No significant absorption was observed above λ_{max} 280nm other than at 229nm in UV spectrophotometer. It indicated that the compound-I did not contain any conjugation.

(ii) Infrared spectroscopy:

The IR spectrum of the compound-I showed absorption at 3421.5 cm^{-1} for hydroxyl group, 2937.4 and 2889.2 cm^{-1} were due to aliphatic -C-H asymmetric and symmetric stretching, respectively. Weak absorption at 1665.5 cm^{-1} indicated the presence of isolated double bond (-CH=CH-) in the compound The absorption at 1458.1 cm^{-1} was due to CH_2 bending vibration. The absorption at 1375.2cm^{-1} was due to geminal dimethyl group.

(iii) ^{1}H NMR Spectroscopy:

The ^{1}H-NMR spectrum of the compound-I revealed the signals at δ 0.687, 0.804, 0.834, 0.921, 1.009, 1.109, and 1.253 ppm due to methyl groups of a steroid. A number of multipletes between 1.442-2.283 ppm were due to methylene and methine protons present in the compound. The broad singlet at 3.523 (H-3) ppm indicated the presence of oxymethine proton flanked with two different methylene groups (-CH_2-CHOH-CH_2-). Two multipletes at 5.151 (1H, m, H-23) and 5.031 (1H, m, H-22) ppm in the spectrum indicated the presence of two olefinic protons attached with two methine groups (>CH-CH=CH-CH<) in side chain of the compound.

A broad singlet at 5.351 (1H, H-6) ppm indicated the presence of a double bond in between a quaternary carbon and a methine carbon i.e. presence of olefinic proton

(iv) ^{13}C-NMR Spectroscopy:

In ^{13}C-NMR spectrum ($CDCl_3$) of compound-1 the signals at 71.819 (C-3) ppm spectrum confirmed the presence of oxymethine group in the compound. The signals at 138.333 (C-22), 129.309 (C-23) ppm spectrum indicated the presence of two olefinic protons attached with two methine groups (>CH-CH=CH-CH<) in the side chain of the compound. Signals at 140.883 (C-5) and121.724 (C-6) ppm in the ^{13}C NMR spectrum indicated the presence of double bond in a quaternary carbon and a methine carbon.

The ^{13}CNMR spectrum exhibited 40 carbon signals, which suggested the compound may be steroids. The DEPT spectrum showed ten methyl carbons which signals at 11.885, 12.009, 12.073, 12.269, 18.809, 19.012, 19.068, 19.420, 19.841, 21.242 ppm fifteen methylene carbons signals at 21.117, 23.101, 24.360, 24.389, 25.427, 26.122, 28.273, 28.936, 31.695, 31.943, 33.980, 37.291, 39.714, 39.809, 42.339 ppm and eleven methine carbons signals at 29.193, 31.902, 36.173, 40.510, 45.87, 50.169, 51.265, 55.991, 56.095, 56.798, 56.896 ppm respectively in the compound. By subtracting these carbon signals from the total ^{13}Cm NMR spectrum, the remaining four signals were assigned to four quaternary carbons. All of the signals were compared with the reported data[52-53] of steroids. It was found that the data fitted well with those of β-sitosterol and stigmasterol. Therefore, the compound-1 was a mixture of β-sitosterol and stigmasterol. This mixture is often isolated from plant sources and they are not easily separated.

Table-9: ^{13}C NMR spectral data of compound 1 (a) & (b) compared with published data[52-53] of β-sitosterol and stigmasterol:

Carbon	Type of carbon	1a	β-Sitosterol (Chemical shift in ppm)	1b	Stigmasterol (chemical shift in ppm)
C-1	CH_2	37.29	37.31	37.291	37.31
C-2	CH_2	31.69	31.57	31.695	31.69
C-3	CH	71.81	71.69	71.819	71.81
C-4	CH_2	42.33	42.25	42.339	42.55
C-5	C	140.88	140.76	140.88	140.5
C-6	CH	121.72	121.59	121.724	121.69
C-7	CH_2	31.90	31.92	31.902	31.94
C-8	CH	31.94	31.92	31.943	31.94
C-9	CH	50.16	50.17	50.169	50.20
C-10	C	36.17	36.51	36.173	36.56
C-11	CH_2	21.11	21.11	21.117	21.11
C-12	CH_2	39.80	39.81	39.714	39.77
C-13	C	42.42	42.33	42.339	42.35
C-14	CH	56.79	56.79	56.896	56.91
C-15	CH_2	24.389	24.32	24.389	24.39
C-16	CH_2	28.27	28.26	28.936	28.96
C-17	CH	56.09	56.11	55.991	56.02
C-18	CH_3	11.88	11.87	12.009	12.07
C-19	CH_3	19.42	19.40	19.420	19.42
C-20	CH	36.17	36.17	40.510	40.54
C-21	CH_3	18.80	18.82	18.809	21.11
C-22	A*	33.98 (-CH_2)	33.95	138.333 (C-H)	138.37
C-23	B*	26.12 (-CH_2)	26.13	129.309 (C-H)	129.69
C-24	CH	45.87	45.55	51.265	51.29
C-25	CH	29.19	29.18	31.943	31.94
C-26	CH_3	19.84	19.84	21.242	21.26
C-27	CH_3	19.06	19.07	19.012	19.02
C-28	CH_2	23.10	23.09	25.427	25.44
C-29	CH_3	12.26	12.32	12.073	12.29

C-22=A*; -CH_2 (1a), -CH (1b)

C-23=B*; -CH_2 (1a), -CH (1b)

(v) Structure of compound I:

According to all the evidences the structure of compound I is determined as a mixture of β-sitosterol and stigmasterol:

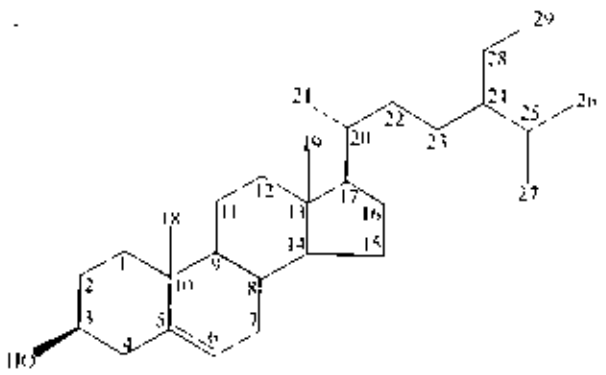


Fig- (12-a): β-Sitosterol

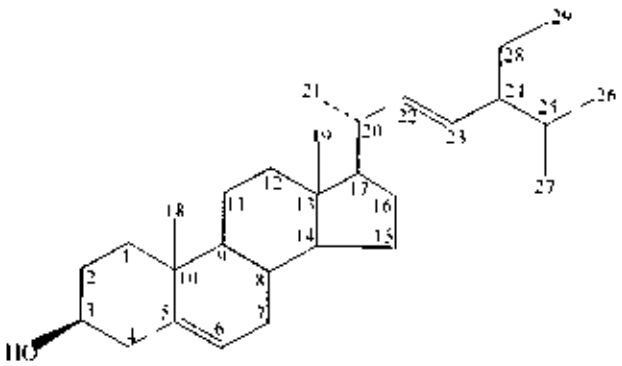


Fig- (12-b): Stigmasterol

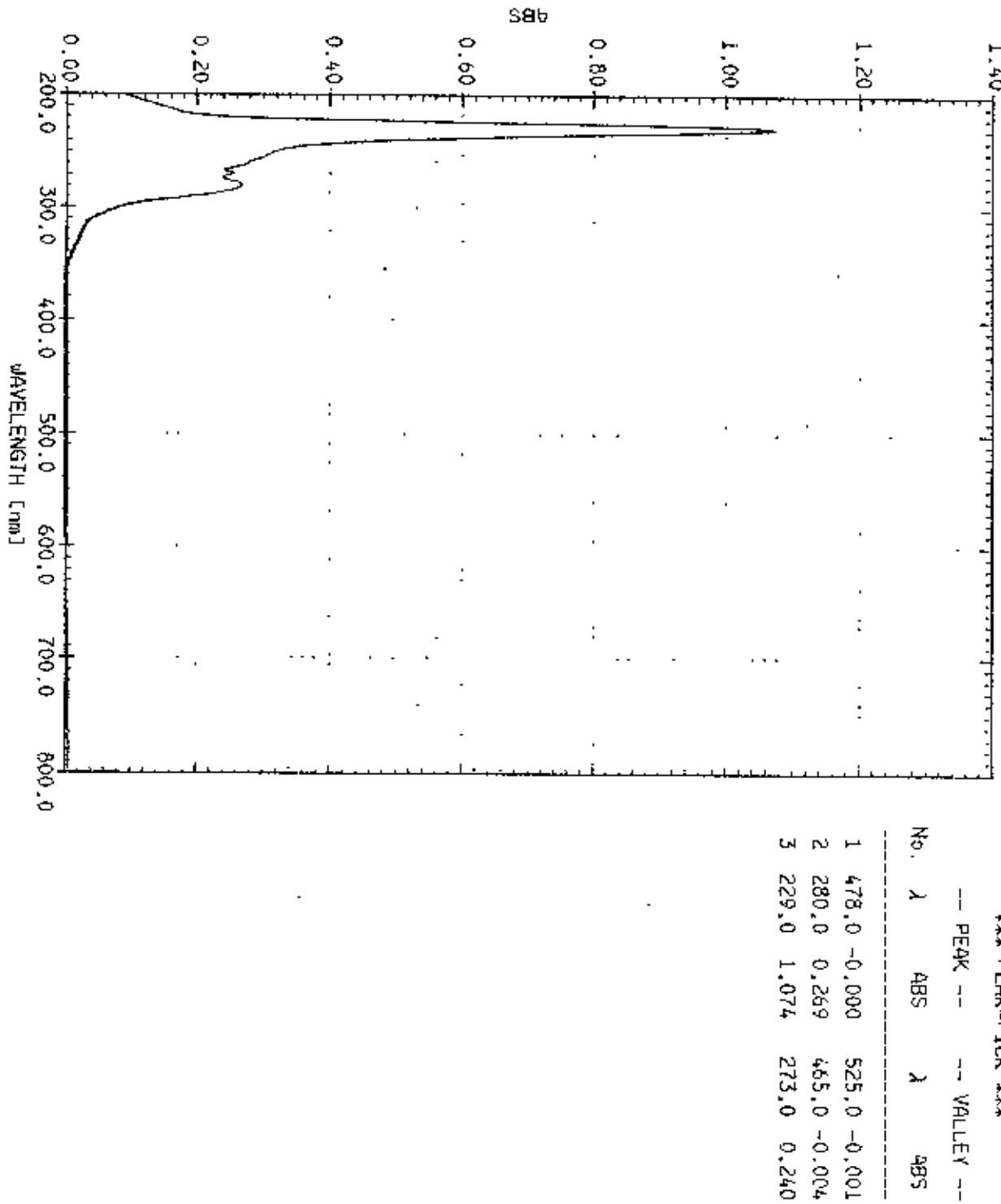
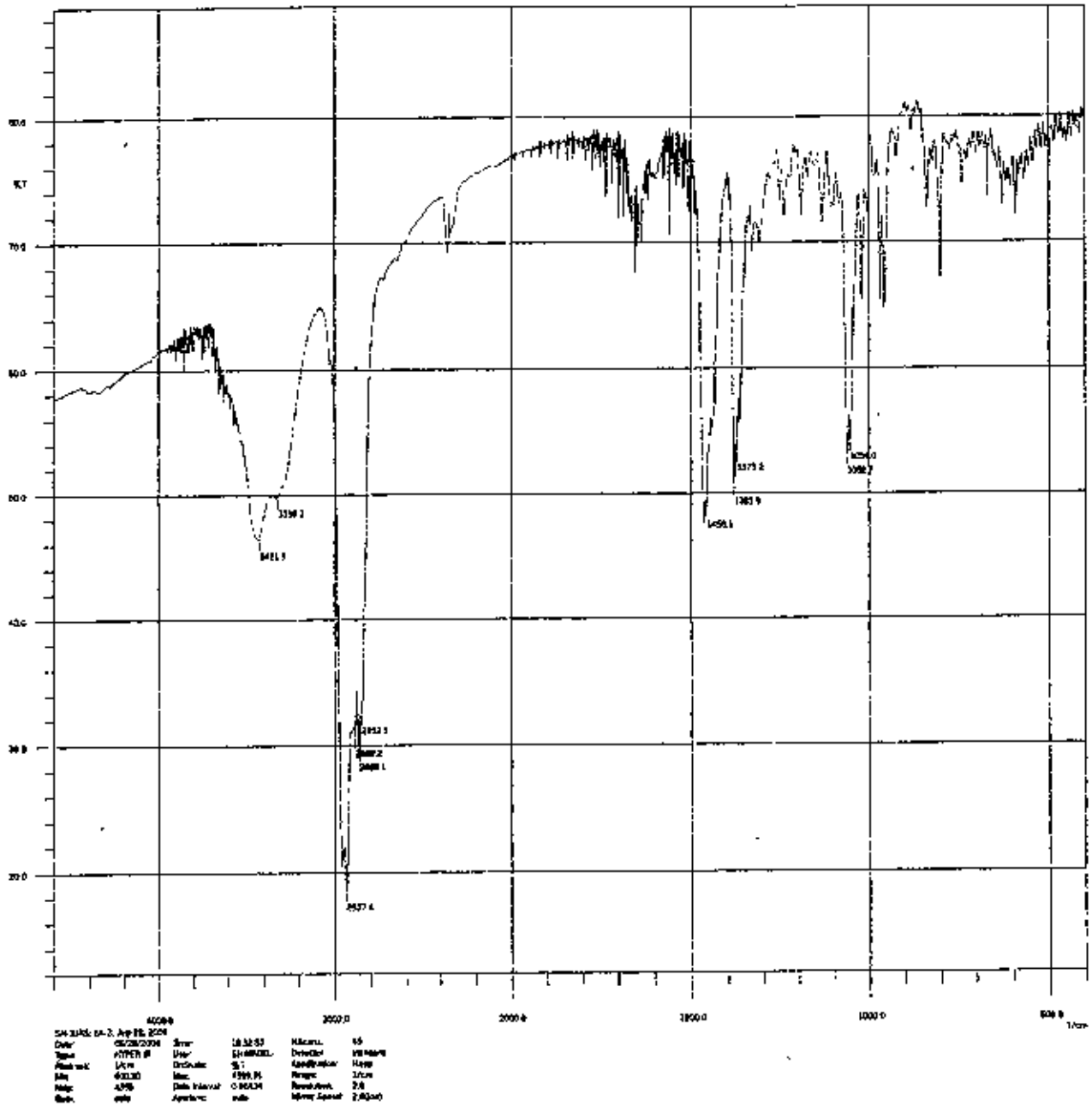


Fig-1: UV spectrum of compound I



Peaktable of SH-3 IR5, 11 Peaks
Threshold, 55, Noise: 2, No Range Selection

No.	Pos. (1/cm)	Inten (%T)
1	1054.0	54.213
2	1062.7	53.016
3	1375.2	53.376
4	1381.9	50.665
5	1458.1	48.797
6	2852.5	32.473
7	2865.1	29.623
8	2889.2	30.657
9	2937.4	18.596
10	3318.3	49.828
11	3421.5	46.401

Sh-3, July 28, 2004

Fig-2: IR spectrum of compound I.

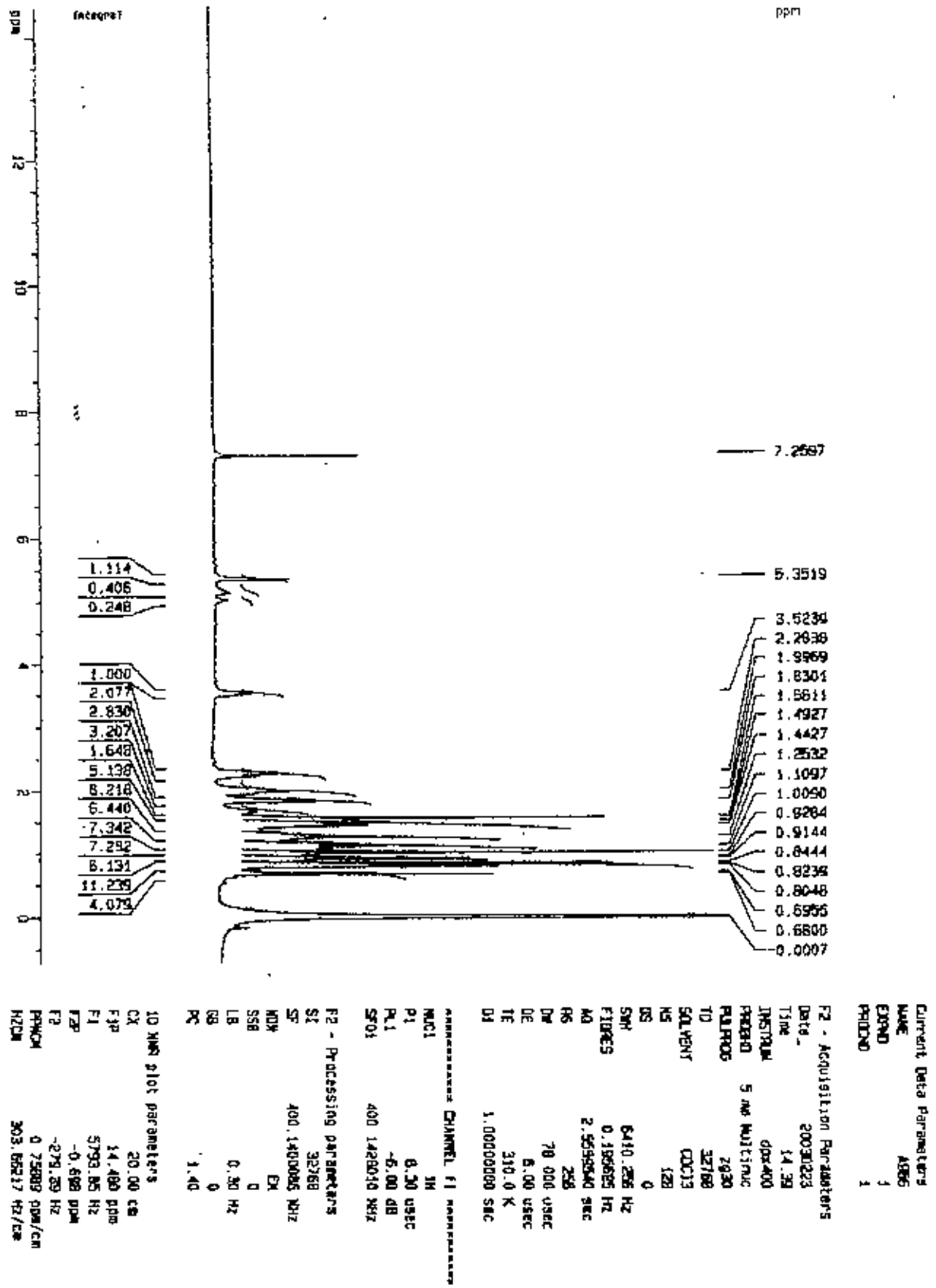


Fig-3: ^{1}H-NMR spectrum of compound I.

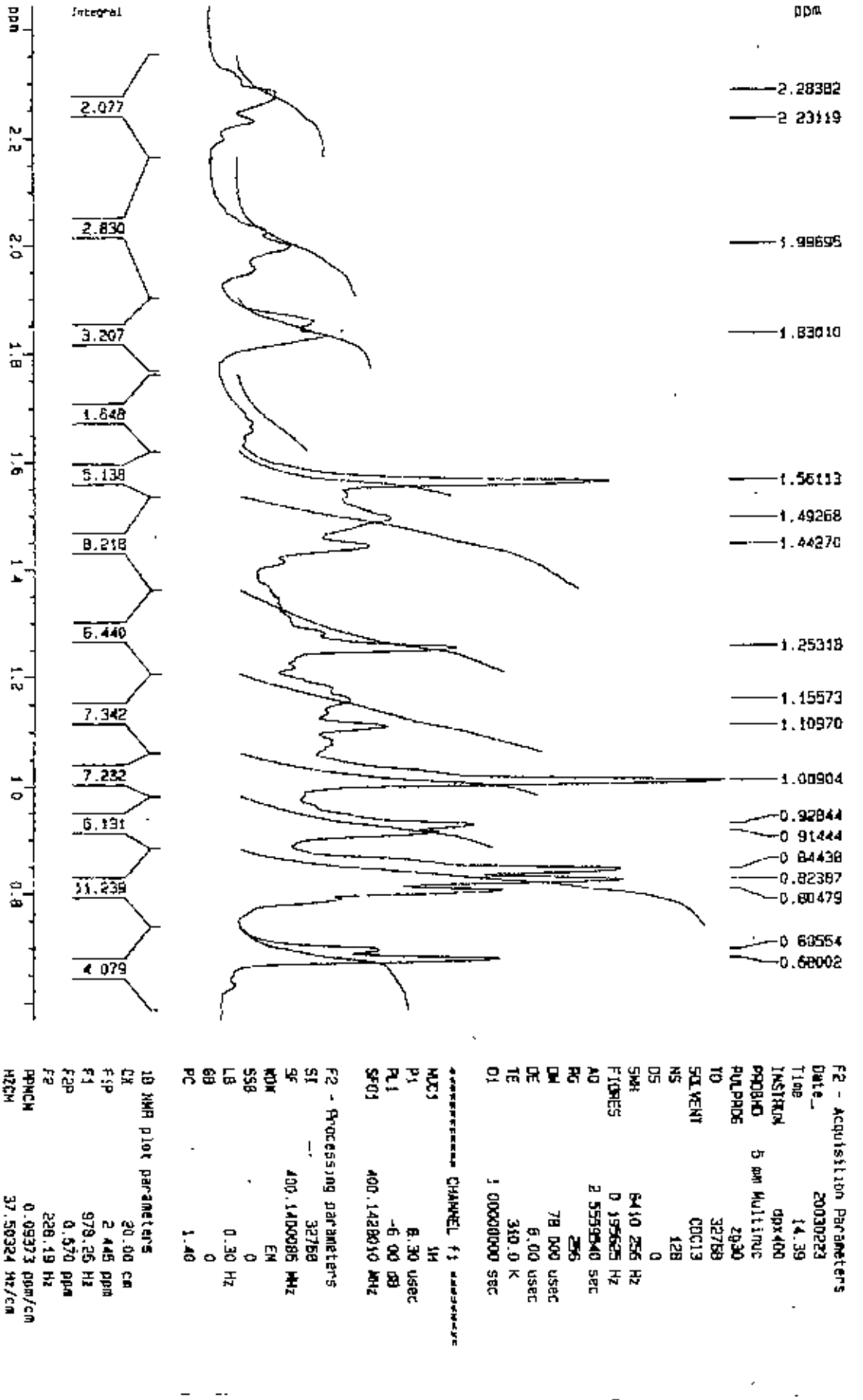
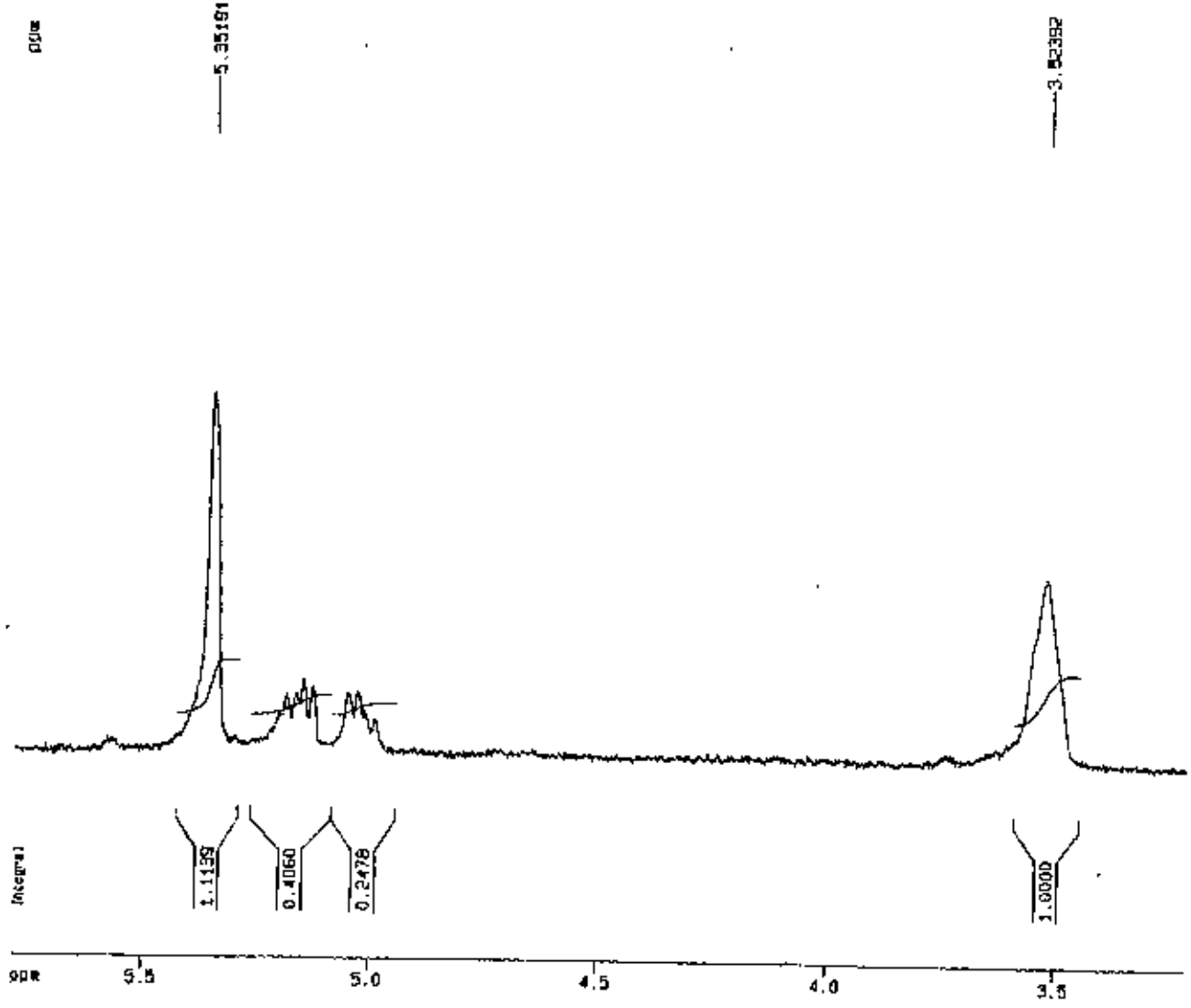


Fig-4: Expansion of ^{1}H-NMR spectrum of compound I.



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PROCNO 1

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PULPROG zg30
TD 32768
SOLVENT CDCl3
NS 128
DS 0
SWH 6410.256 Hz
FIDRES 0.195625 Hz
AQ 2.5559540 sec
RG 256
DW 78.000 usec
DE 6.00 usec
TE 310.0 K
D1 1.00000000 sec

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P1 8.30 usec
PL1 -6.00 dB
SFO1 400.1428010 MHz

F2 - Processing parameters
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SF 400.1400086 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.40

1D NMR plot parameters
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F1P 5.787 ppm
F1 2315.45 Hz
F2P 3.213 ppm
F2 1285.73 Hz
PPMCM 0.12867 ppm/cm
HZCM 51.48521 Hz/cm

Fig-5: Expansion of ^{1}H-NMR spectrum of compound I.

Fig-6: ^{13}C-NMR spectrum of compound I.

Fig-7: Expansion of ^{13}C-NMR spectrum of compound I.

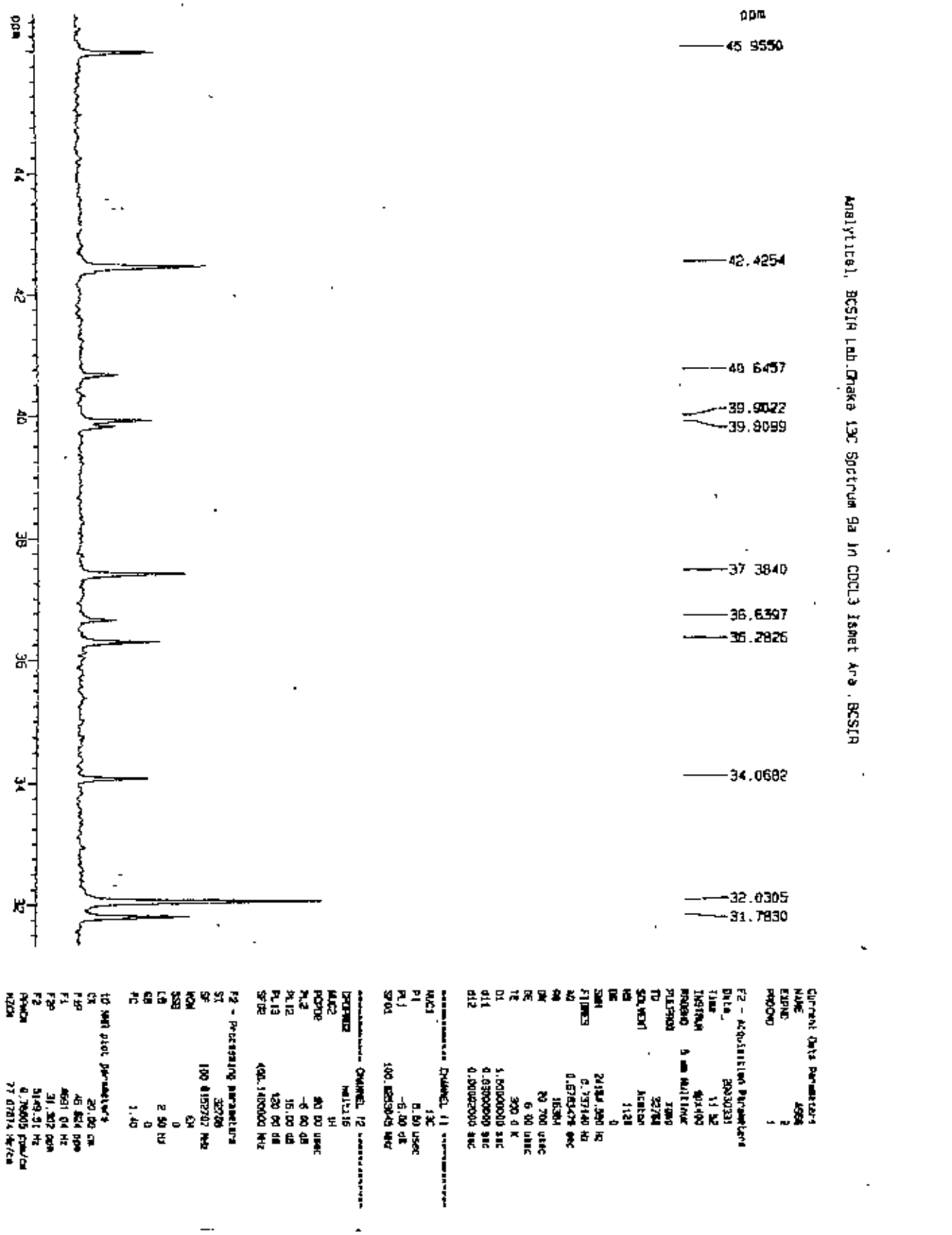

Fig-8: Expansion of ^{13}C-NMR spectrum of compound I.

Fig-9: Expansion of ^{13}C-NMR spectrum of compound I.

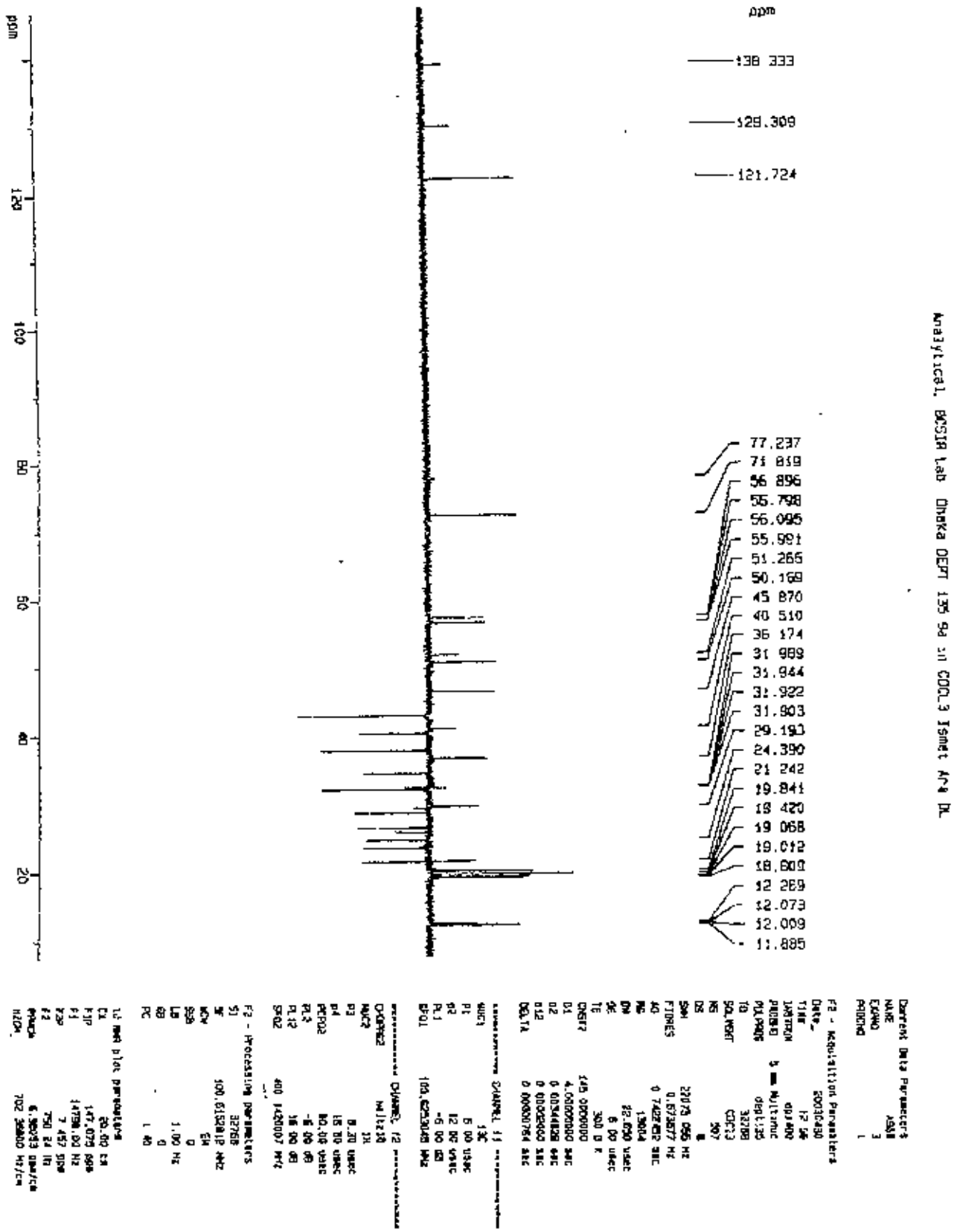

Fig-10: DEPT 135 spectrum of compound I.

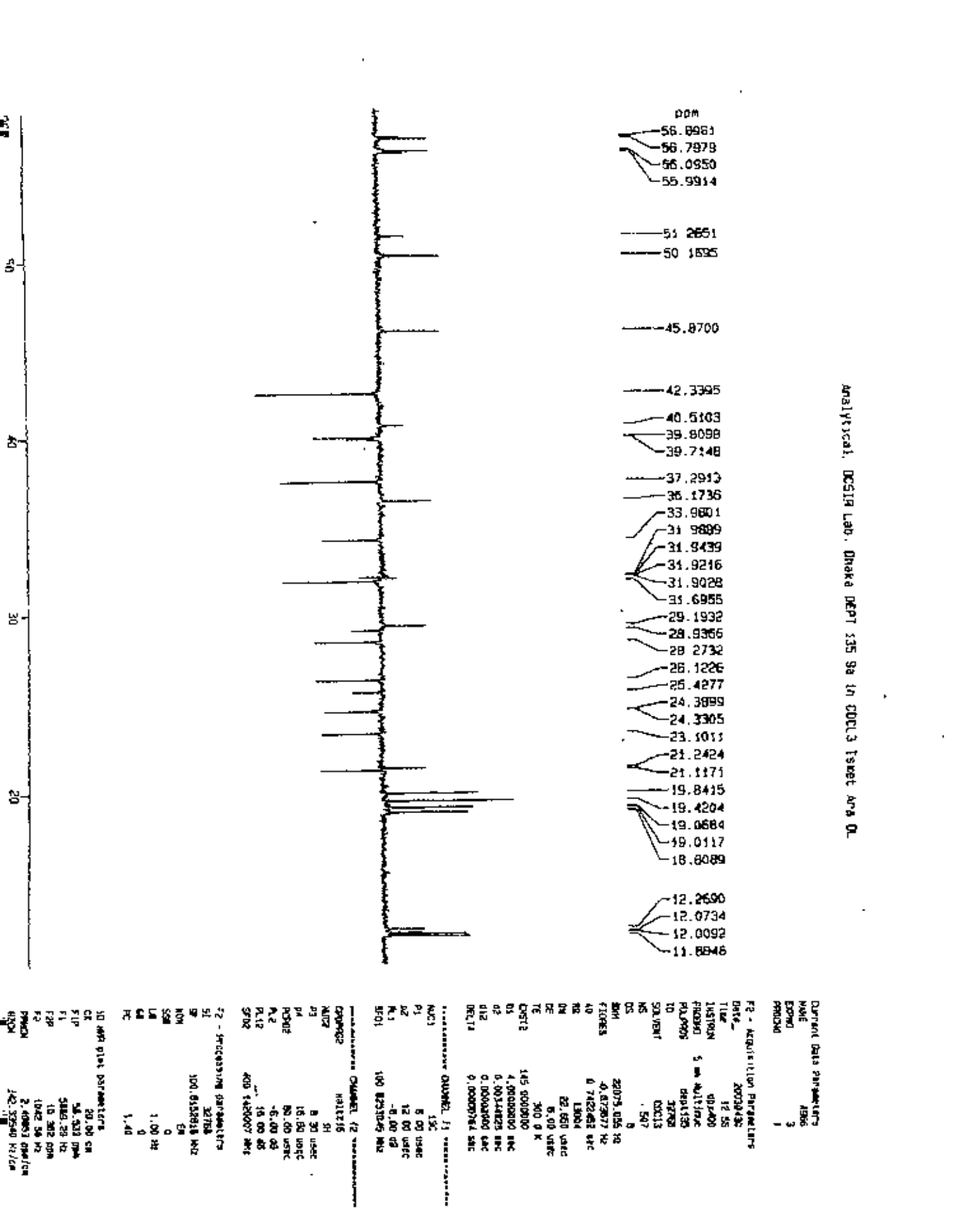

Fig-11: Expansion of DEPT 135 spectrum of compound I.

3.7 Investigation of Ethyl acetate extract:

The dried EtOAc extract was fractionated by using dichloromethane followed by methanol. The eluted fractions were collected in 48 conical flasks and monitored by TLC and were combined on the basis of their R_f values and finally eight fractions (D_1P_1-D_1P_8) were obtained (sec: 2.3) (Table-5). Fraction D_1P_2 gave round spot. It was further washed with n-Hexane for several times to remove associated coloring materials. The residue was crystallized with EtOAc-MeOH mixture. The crystals were dried and compound II was obtained.

3.8 Characterization of compound-II:

3.8.1 Physical characteristics and chemical tests of compound-II:

The compound II was obtained as colourless needles. The crystals were soluble in a mixture of chloroform and methanol. Melting point of the sample was recorded and was found to be 276-278^{0}C. It gave violet color with vanillin-sulphuric acid reagent indicating that compound II might be a triterpene.

3.8.2 Structure elucidation of the compound-II by spectroscopic methods:

(i) Ultraviolet spectroscopy:

The compound-II had an absorption maximum at λ_{max} 229.0 nm and 281.0in MeOH.

(ii) IR spectroscopy:

The IR spectrum of the compound-II had an absorption band at 3459.8 cm^{-1} due to O-H vibrations. Absorption band at 2972.2 cm^{-1} was due to aliphatic -CH_3 stretching. Absorption band at 1685.7 cm^{-1} was due to C=O str. Absorption band at 1451.3 cm^{-1} and 1376.2 cm^{-1} indicated CH_2 and CH_3 bending vibrations respectively. Absorption band in the region 3000-2500 cm^{-1} indicated a carboxylic acid dimer band.

(iii) ^{1}H NMR spectroscopy:

The ^{1}H-NMR spectrum of the compound-II revealed the signals at δ 0.69, 0.76, 0.88, 0.89, 0.90, and 1.62 for six methyl groups. Two broad singlets at 4.532 and 4.661 for the olefinic protons of H-29. A distorted doublet doublet at 3.109 was observed for H-3. A broad singlet at 2.89 was observed for H-19.

Table-10: ^{1}HNMR Splitting pattern of Compound II

Position	Splitting pattern	δ-Value in ppm	Assessment
H-29	S	4.532	1H
H-29	S	4.661	1H
H-3	Distorted doublet doublet	3.109	1H
H-19	Doublet doublet	2.890	1H
H-25	Singlet	0.69	3H
H-27	Singlet	0.76	3H
H-26	Singlet	0.88	3H
H-23	Singlet	0.89	3H
H-24	Singlet	0.90	3H
H-30	Singlet	1.62	3H

(iv) ^{13}C-NMR spectroscopy:

The ^{13}C-NMR spectrum of Compound II gave 30 signals indicating that the compound contained 30 carbons. By DEPT-135 and 90 experiments and their expansions all the carbons were distinguished. DEPT technique indicated the presence of 6 methyl, 11 methylene, 6 methine, 6 quaternary carbons and one carboxylic group in the compound II.

In the ^{13}C-NMR spectrum a signal at 179.01 ppm indicated the presence of a carboxylic group in the compound II.

The deshielded peak at 150.689 ppm was assigned to the quaternary carbon (>C-, C-20) attached with an olefinic carbon, which was supported by the presence of a signal at 109.425 ppm due to the olefinic carbon (=CH_2). The signal at 78.84 ppm was assigned a methine carbon attached with an alcoholic –OH group (-CH-OH) .

The signals at 38.77, 40.64, 37.13, 42.40, 56.17 and 150.68 were assigned to quaternary carbons. The sharp singlets at 27.84, 15.27, 15.84, 16.02, 14.59 and 19.24 ppm were due to methyl groups attached with quaternary carbons. The signals at 38.69, 27.05, 18.23, 34.29, 20.84, 25 49, 30 55, 32 21, 29.62, 37 07 and 109.42 were due to methylene carbons. The signals at 78.85, 55.32, 50.51, 38.26, 46.92 and 49.18 were for methine carbons.

Comparing UV, IR, ^{1}H NMR, and ^{13}C-NMR spectral data[54-56] with the literature value of reported compounds the following structure was elucidated as betulinic acid [3-hydroxy-20 (29)-lupen-28-oic acid].

(v) Structure of compound II:

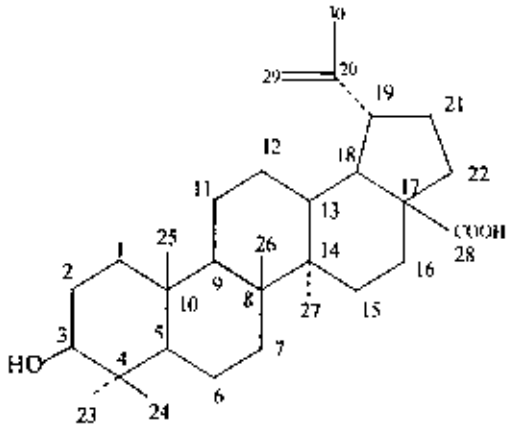

Fig: (22) Betulinic acid

(vi) Comparison of compound-II with reference betulinic acid:

Molecular formula of betulinic acid is: $C_{30}H_{48}O_3$

Tentative molecular formula of Compound II is: $C_{30}H_{48}O_3$

Melting point of betulinic acid is: 275-278^{0}C

Melting point of Compound-II is: 275-278^{0}C

Finally the ^{13}C-NMR data of compound II was compared with ^{13}C-NMR data of known triterpene compound published earlier. It was found that the data fitted very well with the published data of betulinic acid. The comparison of the ^{13}C-NMR signals of Compound II and betulinic acid is given in Table-11.

Table-11: The comparison between ^{13}C-NMR spectral data[54-56] of betulinic acid and the compound II.

Carbon number	Types of carbon	Chemical shift in ppm	
		Compound II	Betulinic acid
C-1	$-CH_2-$	38.69	38.7
C-2	$-CH_2-$	27.05	27.4
C-3	>CH-	78.85	78.9
C-4	>C<	38.77	38.8
C-5	>CH-	55.32	55.3
C-6	$-CH_2-$	18.23	18.3
C-7	$-CH_2$	34.29	34.3
C-8	>C<	40.64	40.7
C-9	>CH-	50.51	50.5
C-10	>C<	37.13	37.2
C-11	$-CH_2$	20.84	20.8
C12	$-CH_2-$	25.49	25.5
C-13	>CH-	38.26	38.4
C-14	>C<	42.40	42.4
C-15	$-CH_2-$	30.55	30.5
C-16	$-CH_2$	32.21	32.1
C-17	>C<	56.17	56.3
C-18	>CH-	46.92	46.8
C-19	>CH-	49.18	49.2
C-20	>C<	150.68	150.3
C-21	$-CH_2-$	29.62	29.7
C-22	$-CH_2-$	37.07	37.0
C-23	$-CH_3$	27.84	27.9
C-24	$-CH_3$	15.27	15.3
C-25	$-CH_3$	15.84	16.0
C-26	$-CH_3$	16.02	16.1
C-27	$-CH_3$	14.59	14.7
C-28	-COOH	179.01	180.5
C-29	$-CH_2-$	109.42	109.6
C-30	$-CH_3$	19.24	19.4

From the above discussion it was confirmed that the compound II was betulinic acid ([3-hydroxy-20 (29)-lupen-28-oic acid], structure, fig-22).

Fig-13: UV spectrum of compound II

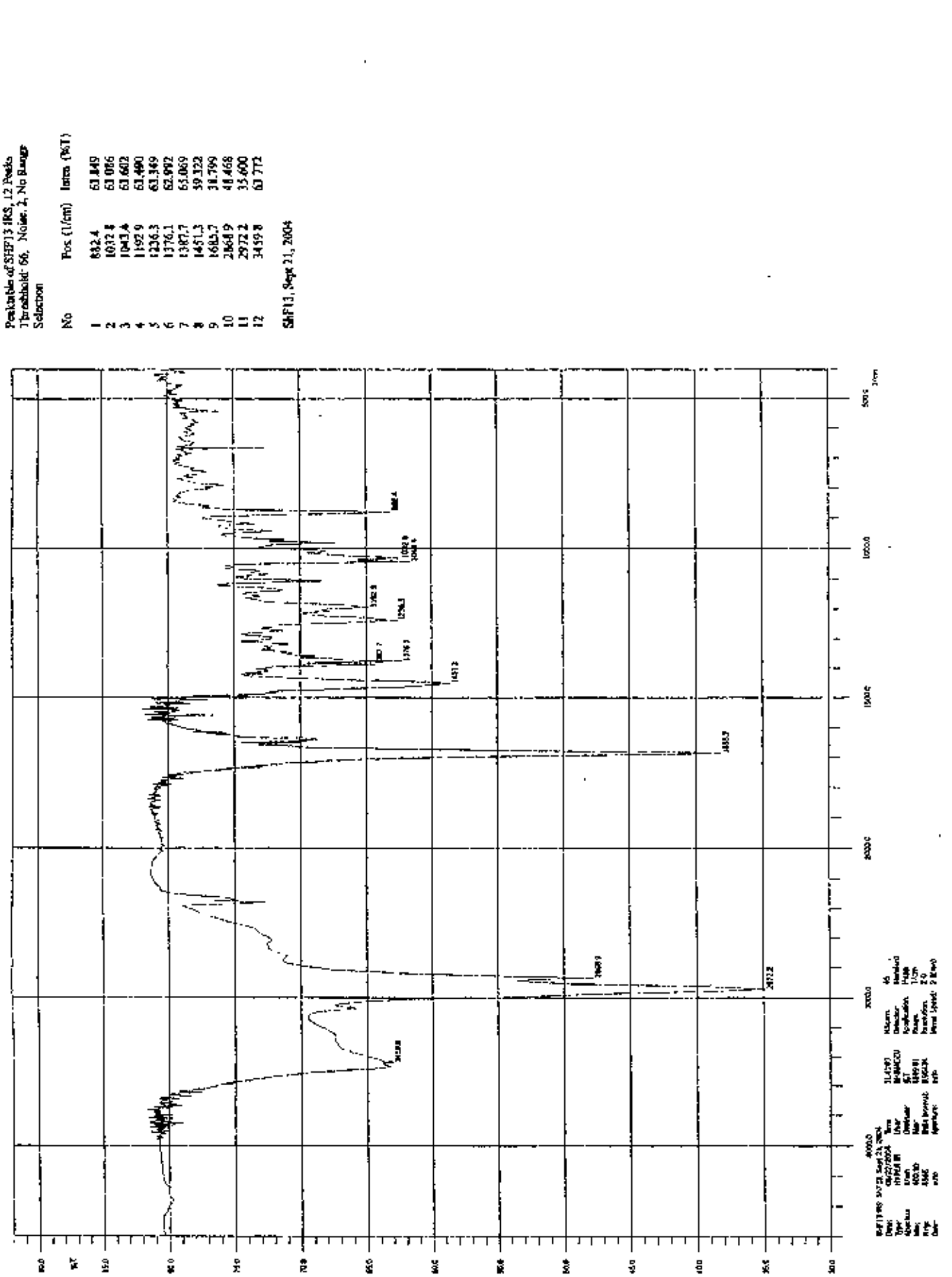

Fig-14: IR spectrum of compound II.

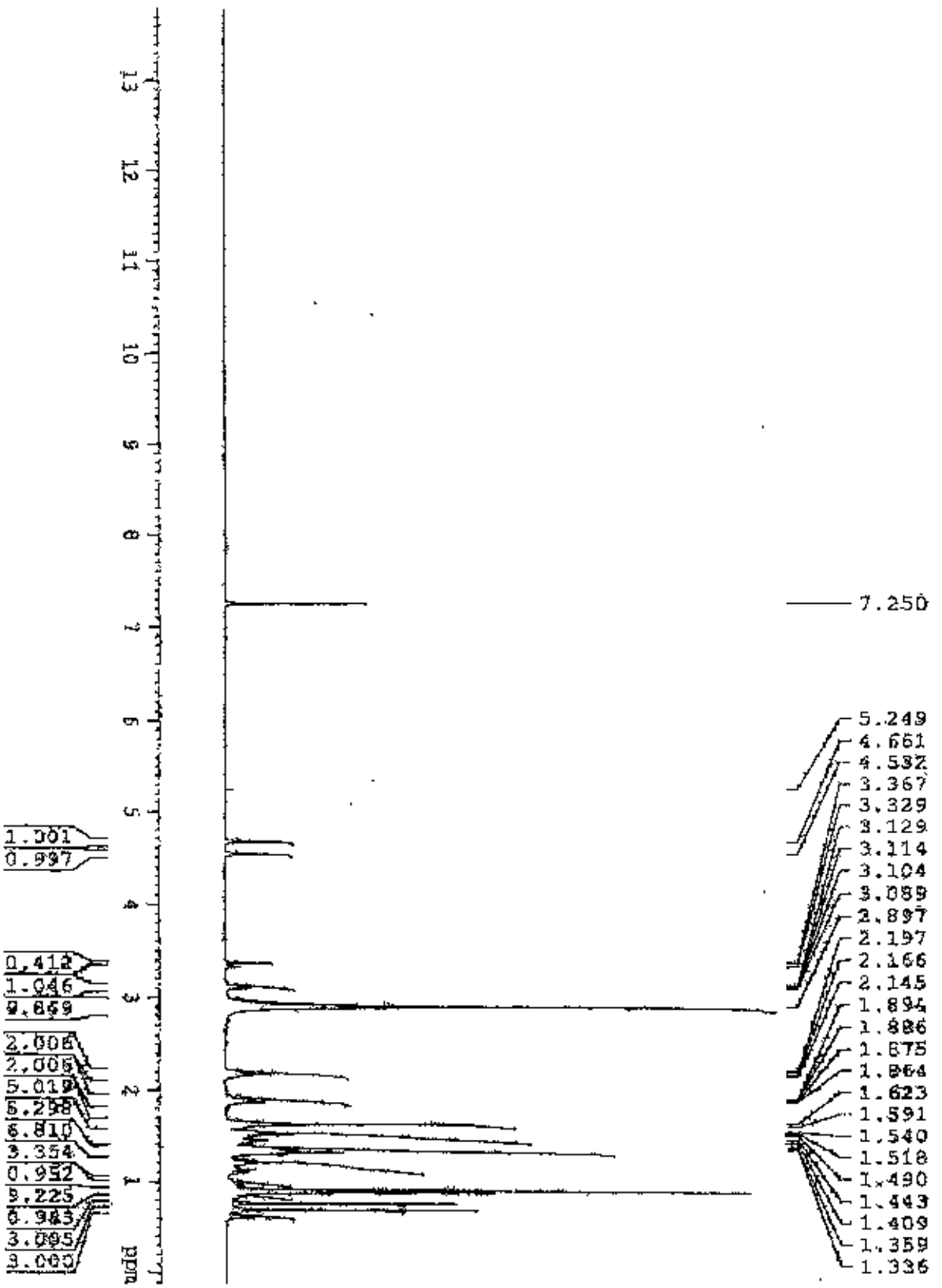

Fig-15: ^{1}H-NMR spectrum of compound II.

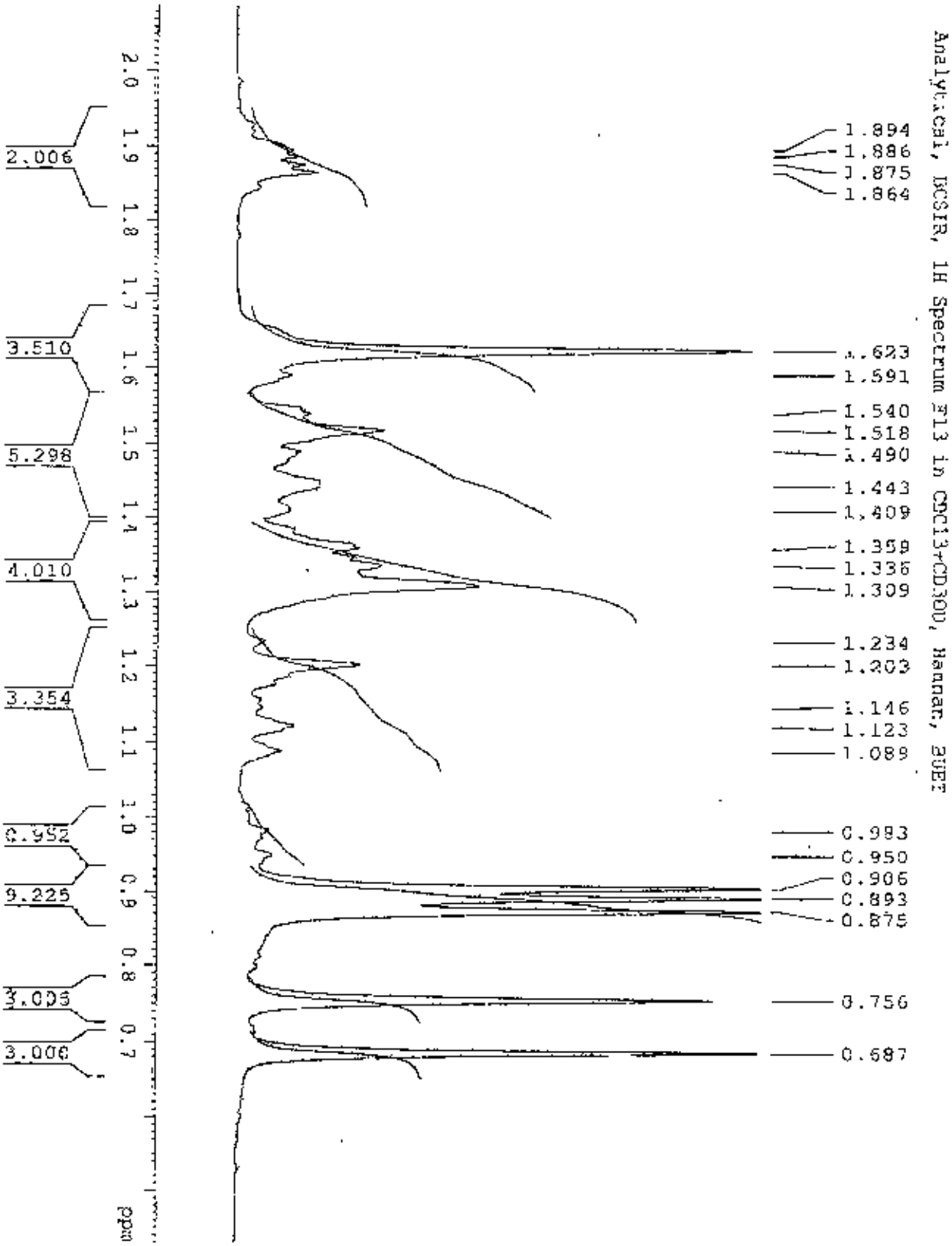

Fig-16: Expansion of ^{1}H-NMR spectrum of compound II.

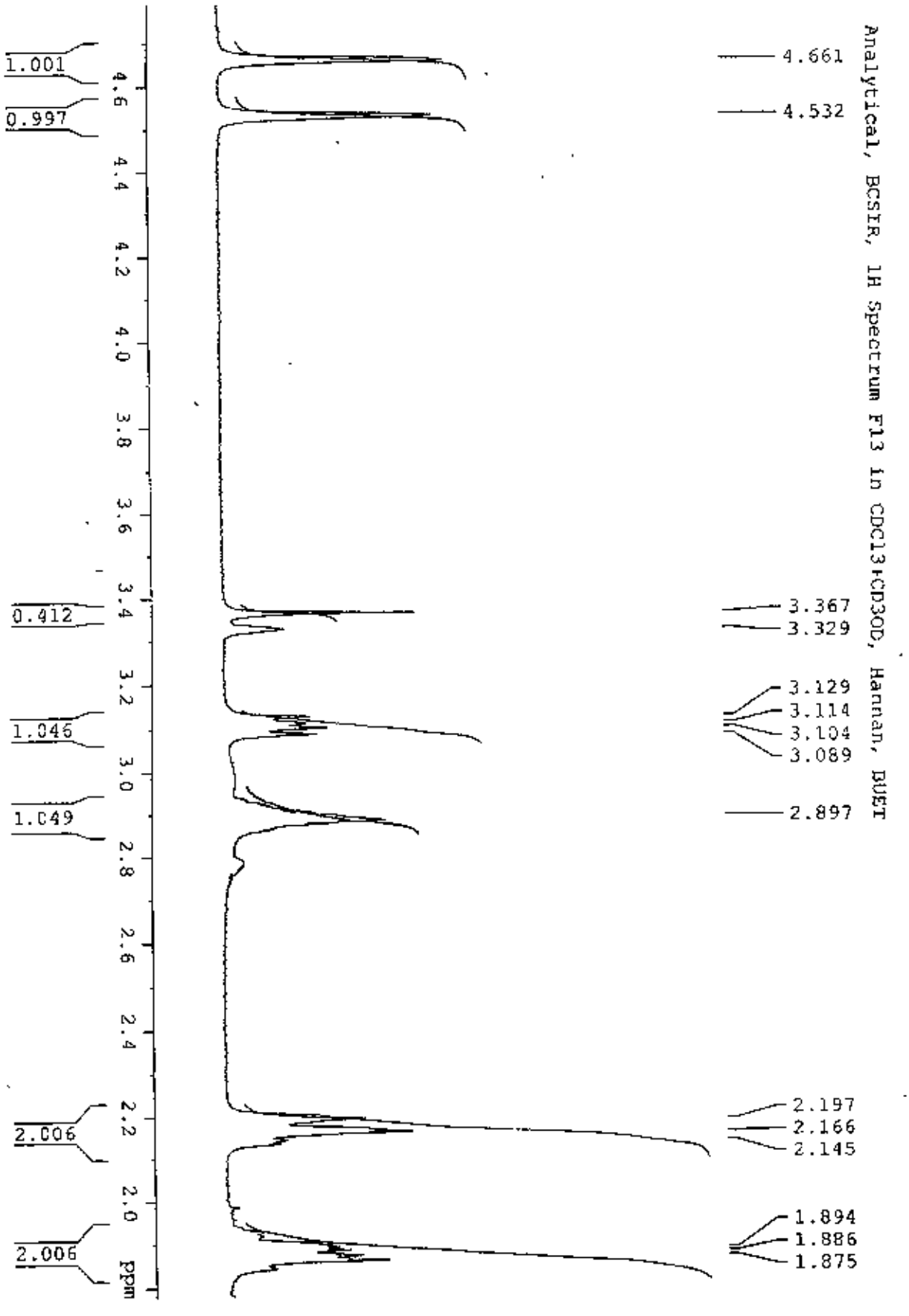

Fig-17: Expansion of ^{1}H-NMR spectrum of compound II.

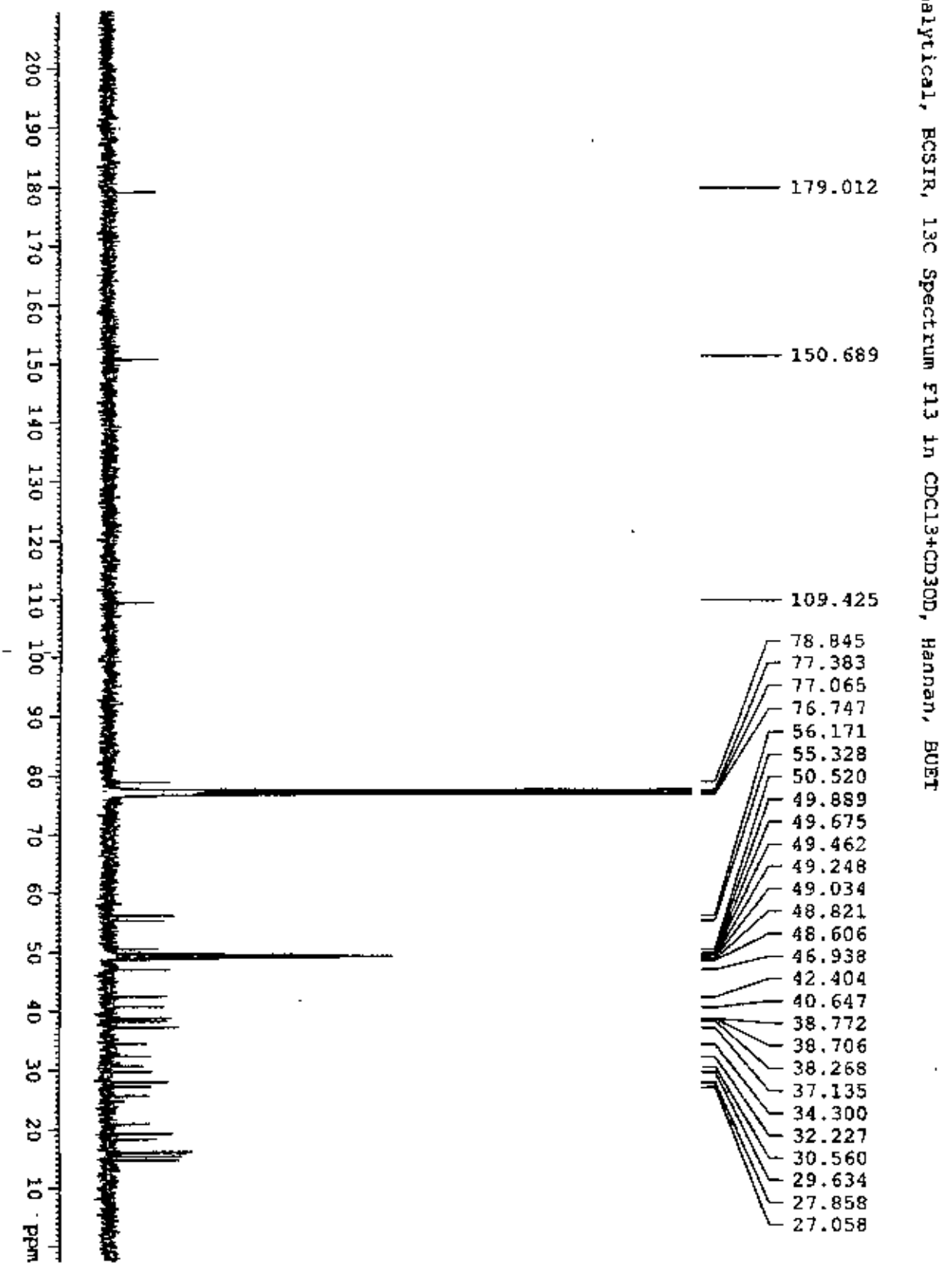

Fig-18: ^{13}C-NMR spectrum of compound II.

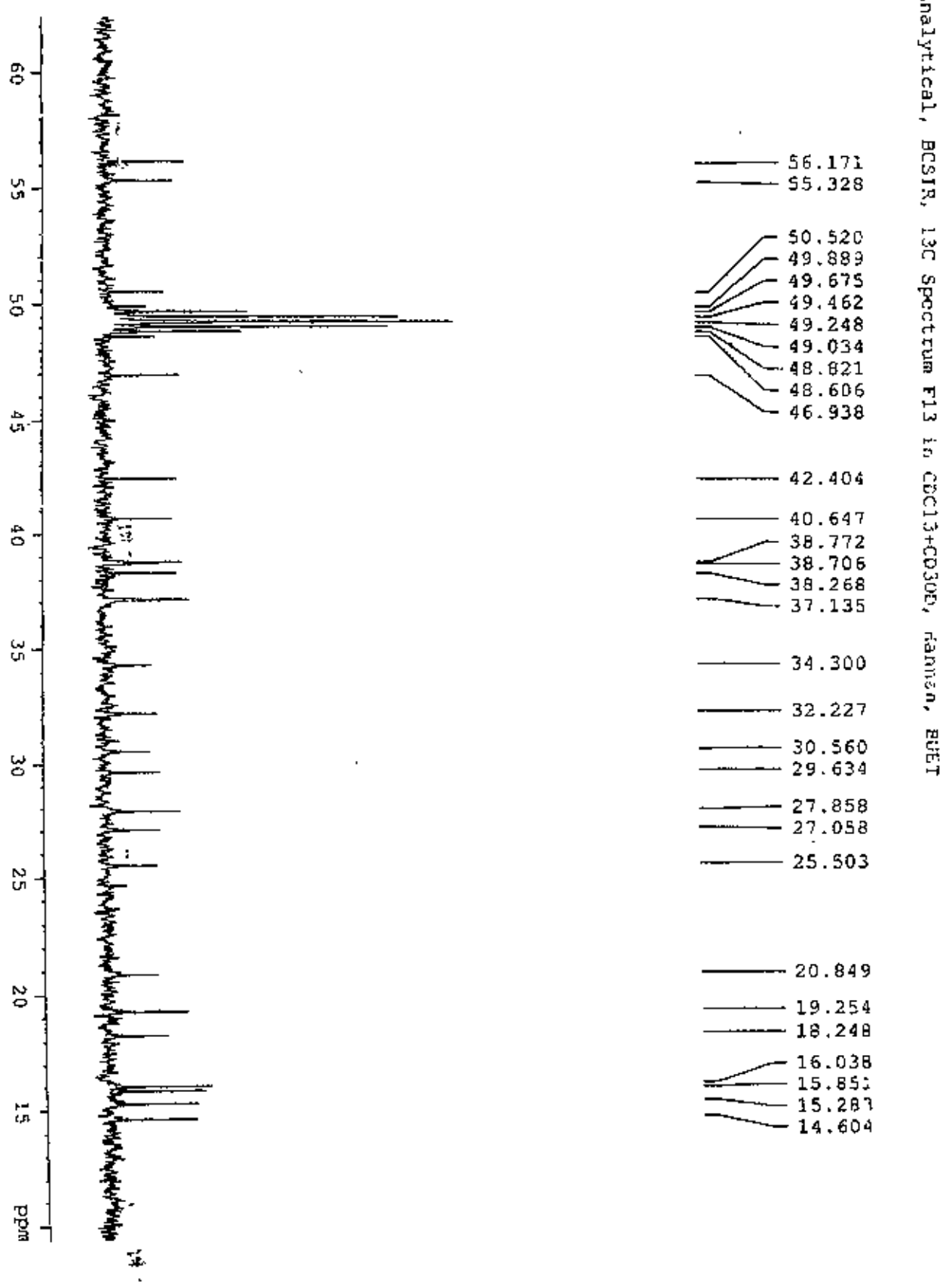

Fig-19: Expansion of ^{13}C-NMR spectrum of compound II.

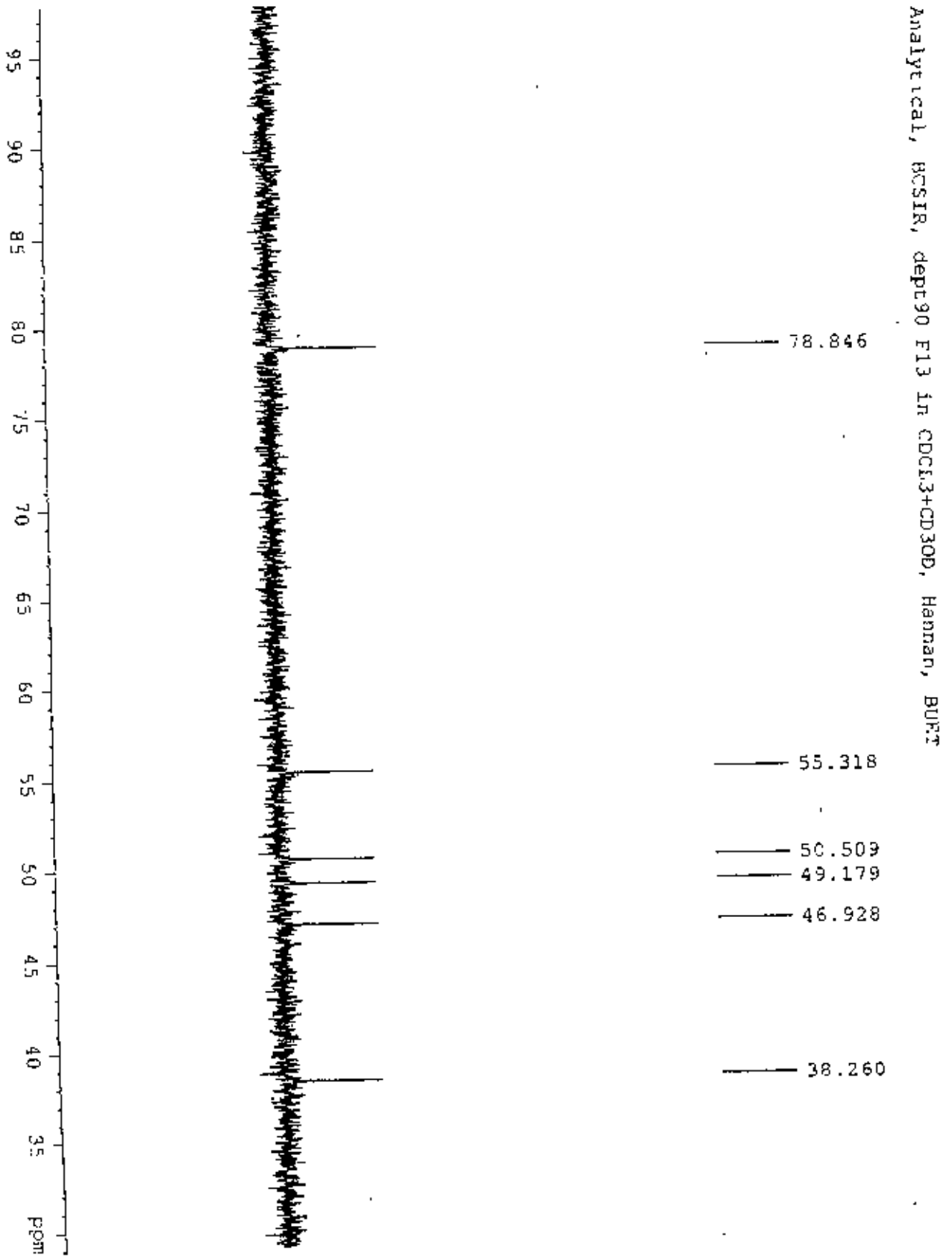

Fig-20: ^{13}C-NMR spectrum of compound II.

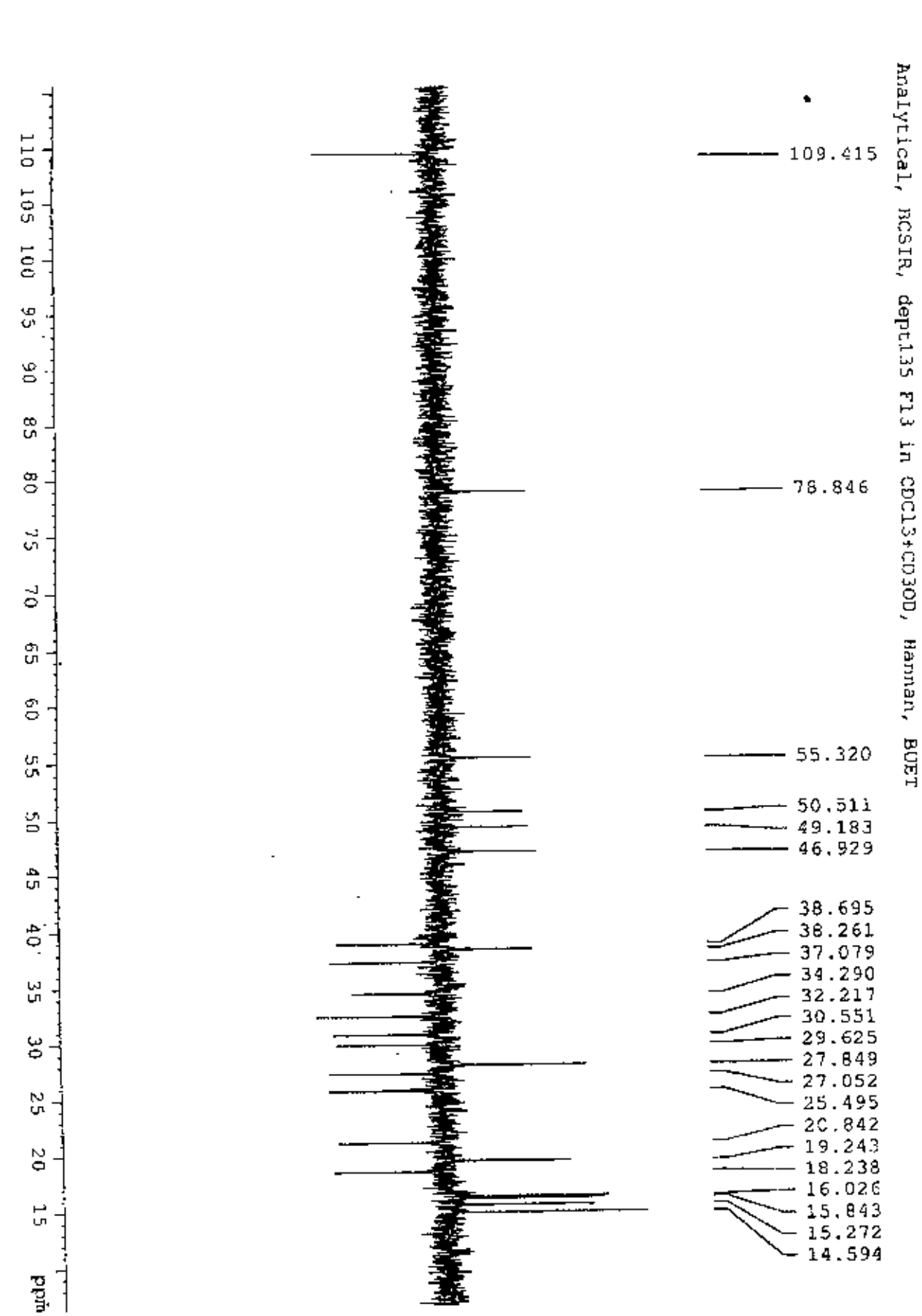

Fig-22: DEPT 135 spectrum of compound II.

3.9 Investigation of D_1P_4:

The fraction D_1P_4 was further fractionated by EtOAc and the polarity of the solvent was gradually increased by the addition of methanol. The eluted samples were collected in different conical flasks and five different fractions were obtained according to their R_f values on TLC plates. Fraction D_2P_3 gave round spot. It was further washed with n-hexane for several times to remove associated coloring materials. The residue was crystallized with EtOAc-MeOH mixture. The crystals were dried and compound III was obtained

3.10 Characterization of compound-III:

3.10.1 Physical characteristics and chemical tests of compound-III:

Compound III was soluble in a mixture of chloroform and methanol. Melting point of the sample was found to be 277-279^{0}C. It gave violet color with vanillin-sulphuric acid reagent indicating that compound III might be a triterpene.

3.10.2 Structure elucidation of the compound-III by spectroscopic methods:

(i) UV spectroscopy:

The compound-III had an absorption maximum at λ_{max} 228.0 nm in MeOH.

(ii) IR Spectroscopy:

The IR (KBr pellet) spectrum of the compound III had major absorbance at 2939.3 cm^{-1} for -CH_3 str. 1735.8 cm^{-1} for ester C=O str. 1365.5 cm^{-1} and 1245.9 cm^{-1} for C-H str.

(iii) ^{1}H NMR spectroscopy:

The ^{1}H-NMR spectrum of the compound-III revealed the signals at δ 1.674, 0.778, 0.827, 0.846, 0.836, 0.93 and 1.022 for seven methyl groups. Two broad singlets at 4.674 and 4.558 for the olefinic protons of H-29. A singlet at 2.027 was observed for methyl group of acetate (H-32). A multiplete at 4.44 was observed for H-3.

(iv) ^{13}C-NMR Spectroscopy:

The ^{13}C-NMR spectrum of Compound III gave 32 signals indicating that the compound contained 32 carbons. By DEPT-135 and their expansions all the

carbons were distinguished. DEPT technique indicated the presence of 8 methyl, 11 methylene, 6 methine, 6 quaternary carbons in the compound III

In the ^{13}C-NMR spectrum a signal at 171.01 ppm indicated the presence of a C=O ester group in the compound. The deshielded peak at 150.97ppm was assigned to the quaternary carbon (>C-) attached with an olefinic carbon, which was supported by the presence of a signal at 109.37 ppm due to the olefinic carbon (CH_2). The signal at 81.02 ppm was assigned a methine carbon attached with a C-O group.

The ^{13}C-NMR spectrum of Compound III gave carbon signals at 38.454, 23.762, 81.026, 38.102, 55.451, 18.257, 34.281, 40.90, 50.403, 37.134, 21.003, 25.174, 37.837, 42.874, 27.493, 35.627, 43.030, 48.350, 48.041, 150.974, 29.900, 40.046, 27.979, 16.015, 16.200, 16.513, 14.541, 18.032, 109.378, 19.332, 171.010 and 21.327ppm.

Comparing UV, IR, ^{1}H-NMR, and ^{13}C-NMR spectral data with the literature value of reported compounds[54] the following structure was elucidated as lupeol acetate.

Table-12: The comparison between ^{1}H-NMR Splitting patterns of compound III with reported data[54] (Lupiol acetate):

C/H No.	^{1}H-NMR	^{1}H-NMR (ref)	C/H No.	^{1}H-NMR	^{1}H-NMR (ref)
1.	-	-	18.	-	-
2.	-	-	19.	-	-
3.	4.44	4.47	20.	-	-
4.	-	-	21.	-	-
5.	-	-	22.	-	-
6.	-	-	23.	0.778	0.76
7.	-	-	24.	0.846	0.84
8.	-	-	25.	0.827	0.83
9.	-	-	26.	0.836	0.93
10.	-	-	27.	0.930	0.93
11.	-	-	28.	1.022	0.93
12.	-	-	29a.	4.674	4.67
13.	-	-	29b.	4.558	4.56
14.	-	-	30.	1.674	1.68
15.	-	-	31.	-	-
16.	-	-	32.	2.027	2.01
17.	-	-		-	-

(v) Structure of compound III:

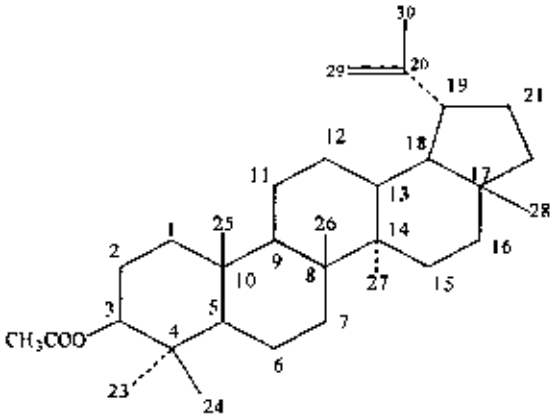

Fig-30: Structure of Lupeol acetate.

(vi) Comparison of compound-III with reference Lupeol acetate:

Molecular formula of Lupeol acetate is: $C_{32}H_{52}O_2$
Tentative molecular formula of Compound III is: $C_{32}H_{52}O_2$
Melting point of Lupeol acetate is: 277-279^{0}C
Melting point of Compound-III is: 277-279^{0}C
Finally the ^{13}C-NMR data of compound III was compared with ^{13}C-NMR data of known triterpene compound published earlier. It was found that the data fitted very well with the published data[54] of Lupeol acetate. The comparison of the ^{13}C-NMR signals of Compound III and Lupeol acetate is given in Table-13.

Table-13: The comparison between ^{13}C-NMR spectral data of compound III with reported data[54] of Lupiol acetate:

C/H No.	Carbon type	13C-NMR δ_C mult.	13C-NMR δ_C mult. Ref	C/H No.	Carbon type	13C-NMR δ_C mult.	13C-NMR δ_C mult. Ref
1.	$-CH_2-$	38.454	38.3	**17.**	>C<	43.030	43.0
2.	$-CH_2-$	23.762	23.7	**18.**	>CH-	48.350	48.2
3.	>CH-	81.026	80.9	**19.**	>CH-	48.041	48.0
4.	>C<	38.102	38.0	**20.**	>C<	150.974	151.0
5.	>CH-	55.451	55.3	**21.**	$-CH_2-$	29.900	29.8
6.	$-CH_2-$	18.257	18.2	**22.**	$-CH_2-$	40.046	40.0
7.	$-CH_2-$	34.281	34.2	**23.**	$-CH_3$	27.98	27.9
8.	>C<	40.90	40.8	**24.**	$-CH_3$	16.015	15.9
9.	>CH-	50.403	50.3	**25.**	$-CH_3$	16.200	16.2
10.	>C<	37.134	37.0	**26.**	$-CH_3$	16.513	16.5
11.	$-CH_2-$	21.003	20.9	**27.**	$-CH_3$	14.541	14.5
12.	$-CH_2-$	25.174	25.0	**28.**	$-CH_3$	18.032	18.0
13.	>CH-	37.837	37.8	**29.**	$-CH_2-$	109.378	109.3
14.	>C<	42.874	42.8	**30.**	$-CH_3$	19.332	19.3
15.	$-CH_2-$	27.493	27.4	**31.**	>C<	171.010	170.6
16.	$-CH_2-$	35.627	35.5	**32.**	$-CH_3$	21.327	21.5

From the above discussion it was confirmed that the compound III was Lupeol acetate (Structure, fig-30).

Fig-23: UV spectrum of compound III

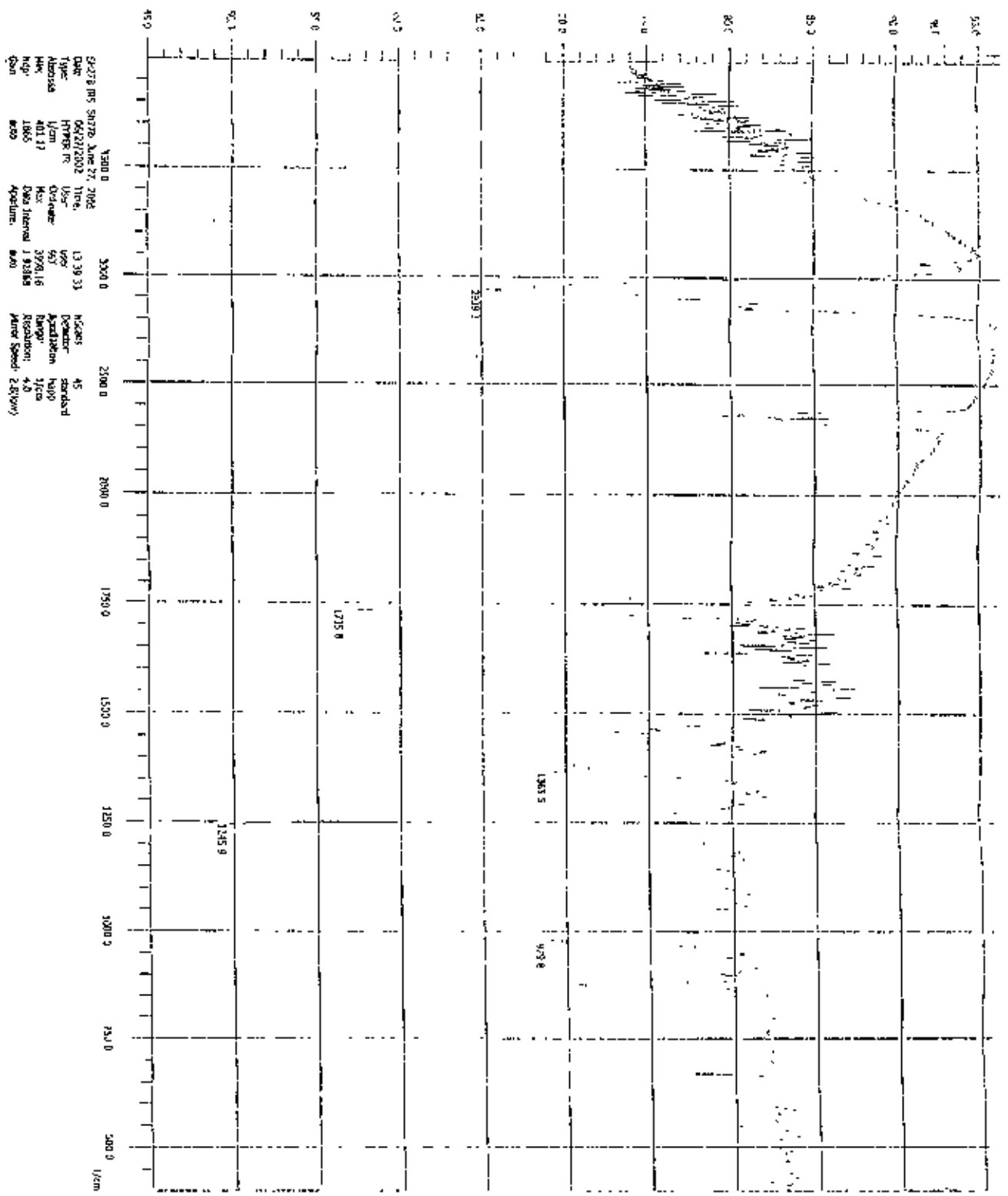

Fig-24: IR spectrum of compound III

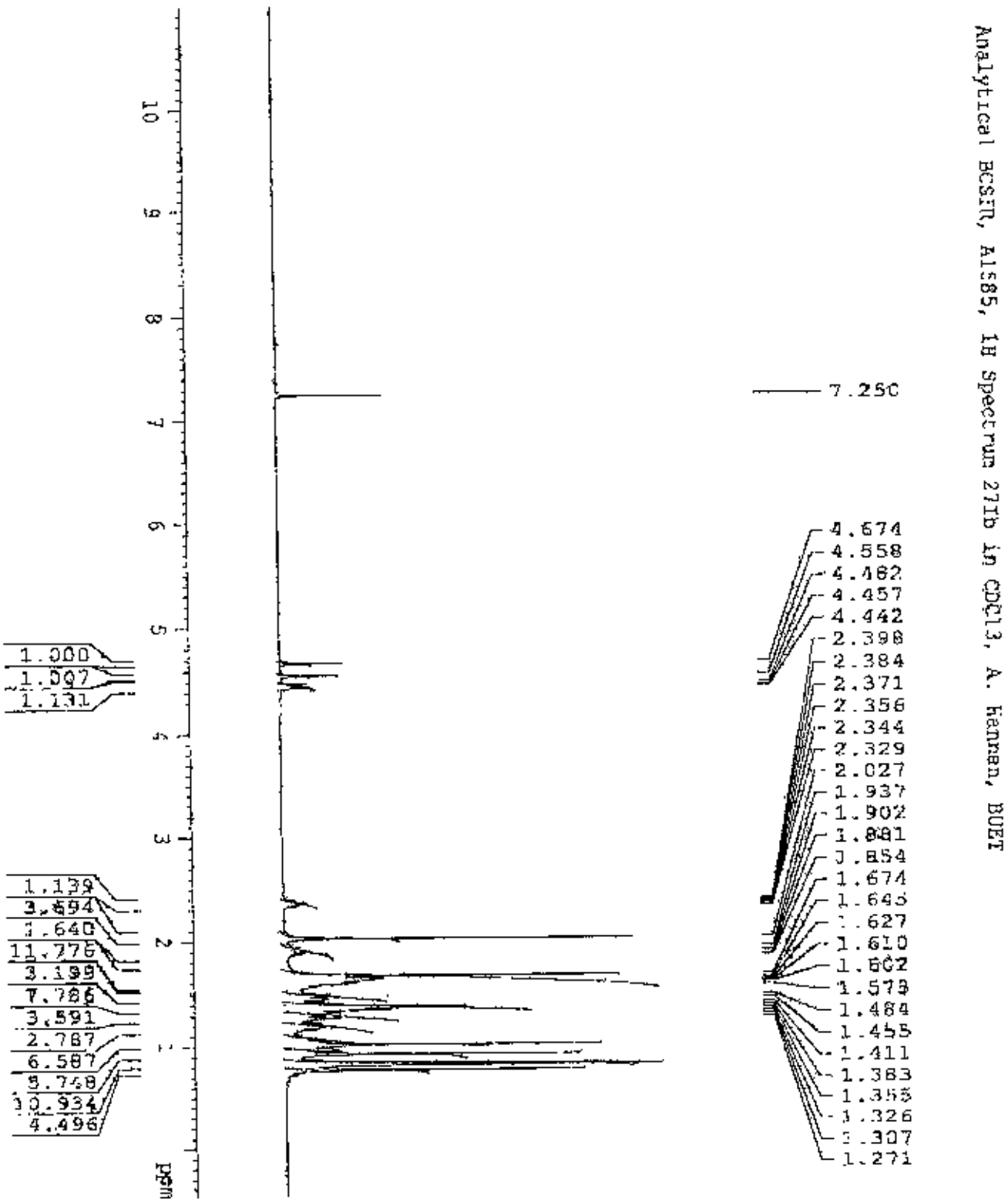

Fig-25: ^{1}H-NMR spectrum of compound III.

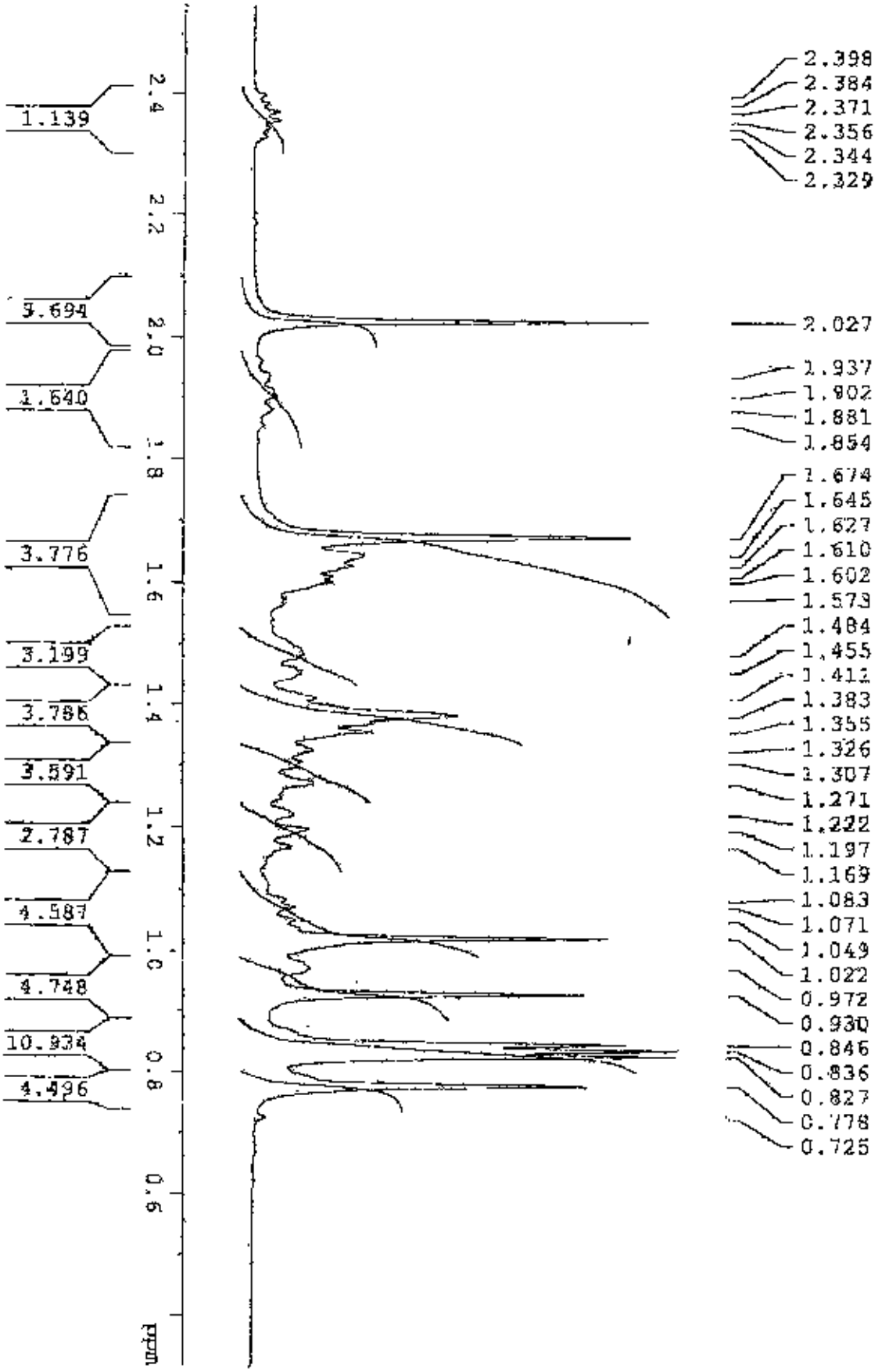

Fig-26: Expansion of ^{1}H-NMR spectrum of compound III.

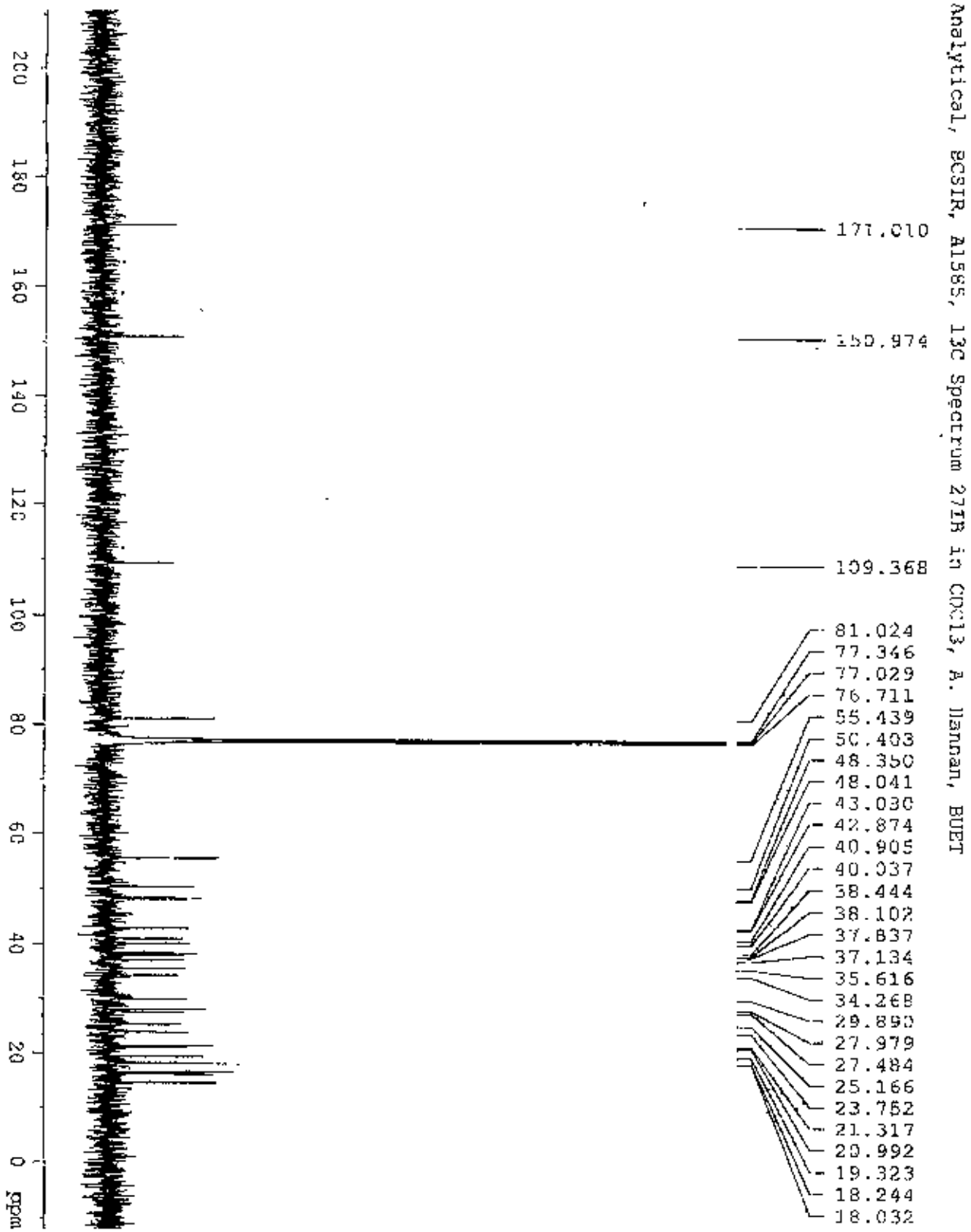

Fig-27: ^{13}C-NMR spectrum of compound III.

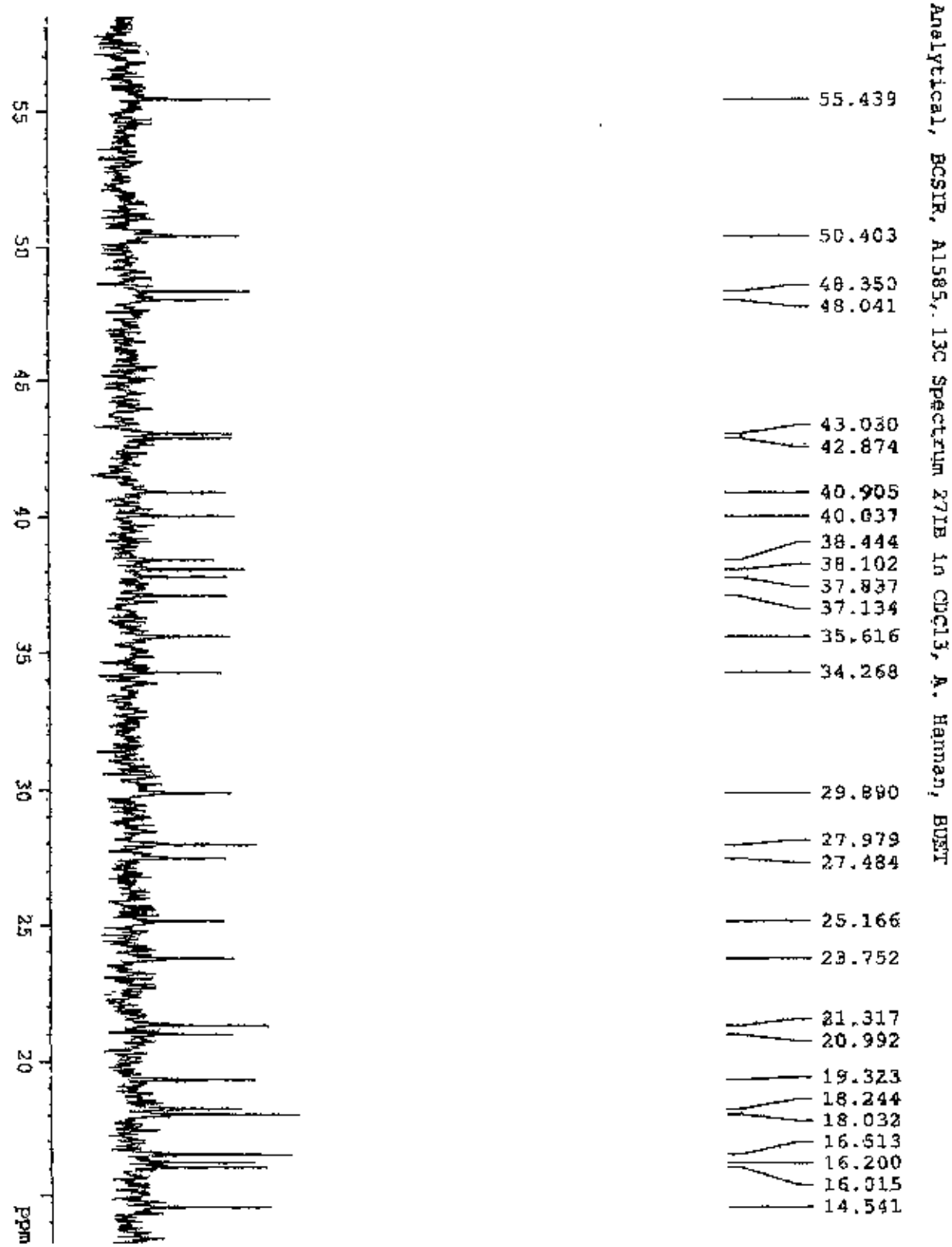

Fig-28: Expansion of ^{13}C-NMR spectrum of compound III.

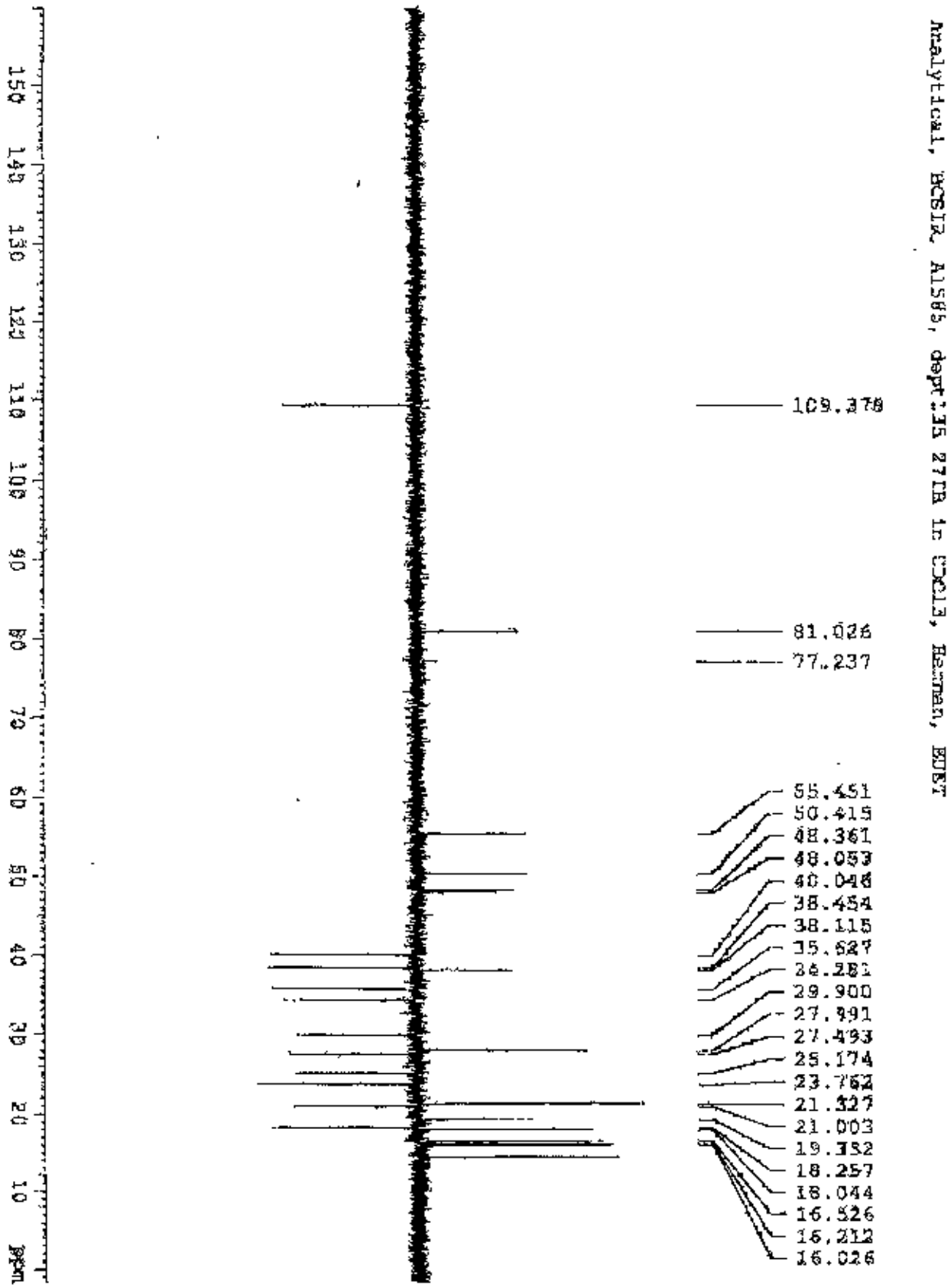

Fig-29: DEPT 135 spectrum of compound III.

3.11 Investigation of 1-Butanol extract:

The 1-Butanol extract was fractionated by a Sephadex LH-20 gel column with solvents water and methanol. The eluted fractions were collected in different conical flasks. According to the TLC pattern six fractions (D_3P_1-D_3P_6) were obtained. Fraction D_3P_6 gave a spot with small tailing. It was then further fractionated by a silica gel column. The column was first eluted by dichloromethane and the polarity of the solvent was gradually increased by the addition of methanol. The eluted samples were collected in different conical flasks and five different fractions were obtained according to their R_f values on TLC plates. Fraction D_4P_2 gave round spot. It was further washed with n-hexane for several times to remove associated coloring materials. The residue was white solid crystal. The crystals were dried and compound IV was obtained.

3.12 Characterization of compound-IV:

3.12.1 Physical characteristics and chemical tests of compound-IV:

The compound IV was soluble in a mixture of dicholoromethane and methanol. It gave reddish color when tested by Salkawoski method indicating that the compound may be a steroid. Again it developed reddish brown color when tested by phenol-sulphuric acid, indicating that the compound may contain a sugar residue. Melting point of the compound was found to be 178°C.

3.12.2 Structure elucidation of the compound-IV by spectroscopic methods:

(i) Ultraviolet spectroscopy:

The UV spectrum of the compound was recorded and was found that the compound had absorption maximum at λ_{max} 229 nm in methanol.

(ii) Infrared spectroscopy:

The IR (KBr pellet) spectrum of the compound IV had major absorbance at 3565.1 for –OH, 2931.6 cm^{-1} for -CH_3 str, 1652.9 cm^{-1} for C=C str.

(iii) ^{1}H-NMR spectroscopy:

The ^{1}H-NMR of the compound-IV revealed signals at 0.635, 0.752, 0.791, 0.791, 0.868 and 0.964 for methyl groups. A multiplete was observed at 3.20 for H-3. A distorted doublet was observed at 5.30 for H-6. A doublet was observed at 4.35 for the anomeric proton of glucose. Multipletes were observed at 3.34-3.80 for the oxymethine protons of glucose.

(iv) ^{13}C-NMR spectroscopy:

The ^{13}C-NMR spectrum of Compound IV gave 35 signals at 36.544, 29.36, 78.795, 39.34, 121.764, 140.014, 33.761, 31.573, 49.893, 37.192, 20.725, 38.651, 42.521, 56.441, 25.549, 29.245, 55.990, 11.501, 19.334, 36.945, 18.894, 39.652, 22.721, 45.554, 18.561, 28.827, 19.334, 24.201, 11.359, 100.809, 73.251, 76.171, 69.904, 75.556 and 61.482 ppm.

The signal at 78.79 ppm indicated the presence of a deshielded oxymethine group which is supported by the preseoce of a signal at 100.80 for an anomeric group of glucose. Signals at 121.76 (C-5) and 140.01 (C-6) ppm in the ^{13}CNMR spectrum indicated the presence of a double bond in between a quaternary carbon and a methine carbon.

Comparing UV, IR, 1H-NMR, and ^{13}C-NMR spectral data with the literature value of reported compounds[57] the following structure was elucidated as β-sitosterol-3-O-β-D glucoside.

(v) Structure of compound IV

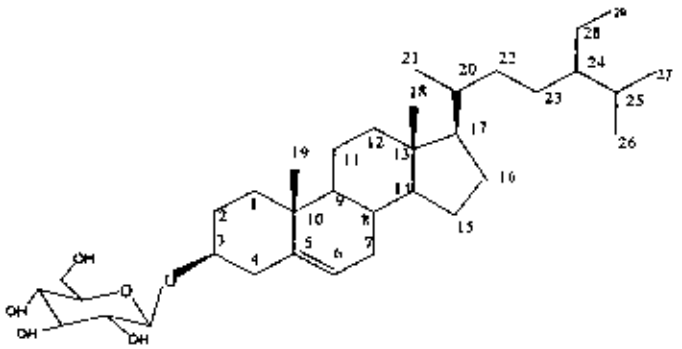

Fig-37: Structure of β-sitosterol glucoside.

(vi) Comparison of compound-IV with reference β-sitosterol glucoside:

Molecular formula of β-sitosterol-3-O-β-D glucoside is: $C_{35}H_{60}O_6$
Tentative molecular formula of Compound IV is: $C_{35}H_{60}O_6$
Melting point of β-sitosterol-3-O-β-D glucoside is: 178^0C
Melting point of Compound-IV is: 178^0C
Finally the ^{13}C-NMR data of compound IV was compared with ^{13}C-NMR data of known steroid compound published earlier. It was found that the data fitted very well with the published data[57] of β-sitosterol-3-O-β-D glucoside. The comparison of the ^{13}C-NMR signals of Compound IV and β-sitosterol-3-O-β-D glucoside is given in Table-14.

Table-14: Comparison of ^{13}C-NMR of Compound IV with reported data (β-sitosterol glucoside)

C/H No.	Carbon type	^{13}C-NMR ppm	^{13}C-NMR ppm (Ref)	C/H No.	Carbon type	^{13}C-NMR ppm	^{13}C-NMR ppm (Ref)
1.	$-CH_2-$	36.544	36.50	19.	$-CH_3$	19.334	19.37
2.	$-CH_2-$	29.36	29.40	20.	>CH-	36.945	36.88
3.	>CH-	78.795	18.99	21.	$-CH_3$	18.894	18.85
4.	$-CH_2-$	39.34	39.51	22.	$-CH_2-$	39.652	39.68
5.	>C<	121.764	121.85	23.	$-CH_2-$	22.721	22.87
6.	>CH-	140.014	140.12	24.	>CH-	45.554	45.69
7.	$-CH_2-$	33.761	33.80	25.	>CH-	18.561	18.61
8.	>CH-	31.573	31.61	26.	$-CH_3$	28.827	28.78
9.	>CH-	49.893	49.99	27.	$-CH_3$	19.334	19.26
10.	>C<	37.192	37.17	28.	$-CH_2$	24.201	24.21
11.	$-CH_2-$	20.725	20.95	29.	$-CH_3$	11.359	11.45
12.	$-CH_2-$	38.651	38.61	C_1	---	100.809	100.91
13.	>C<	42.521	42.24	C_2	---	73.251	73.35
14.	>CH-	56.441	56.58	C_3	---	76.171	76.26
15.	$-CH_2-$	25.549	25.66	C_4	---	69.904	69.99
16.	$-CH_2-$	29.245	29.34	C_5	---	75.556	75.60
17.	>CH-	55.990	55.98	C_6	---	61.482	61.51
18.	$-CH_3$	11.501	11.54				

C_1'-C_6' (Carbon of glucoside)

By analyzing the spectral data UV, IR, ^{1}H-NMR, ^{13}C-NMR of compound IV and a comparative studies with the literature values of the reported compounds[57]. The structure of compound IV was determined as β-sitosterol-3-O-β-D glucoside. Literature showed that this compound was isolated for the first time from the leaves of this plant.

Fig-21: UV spectrum of compound IV

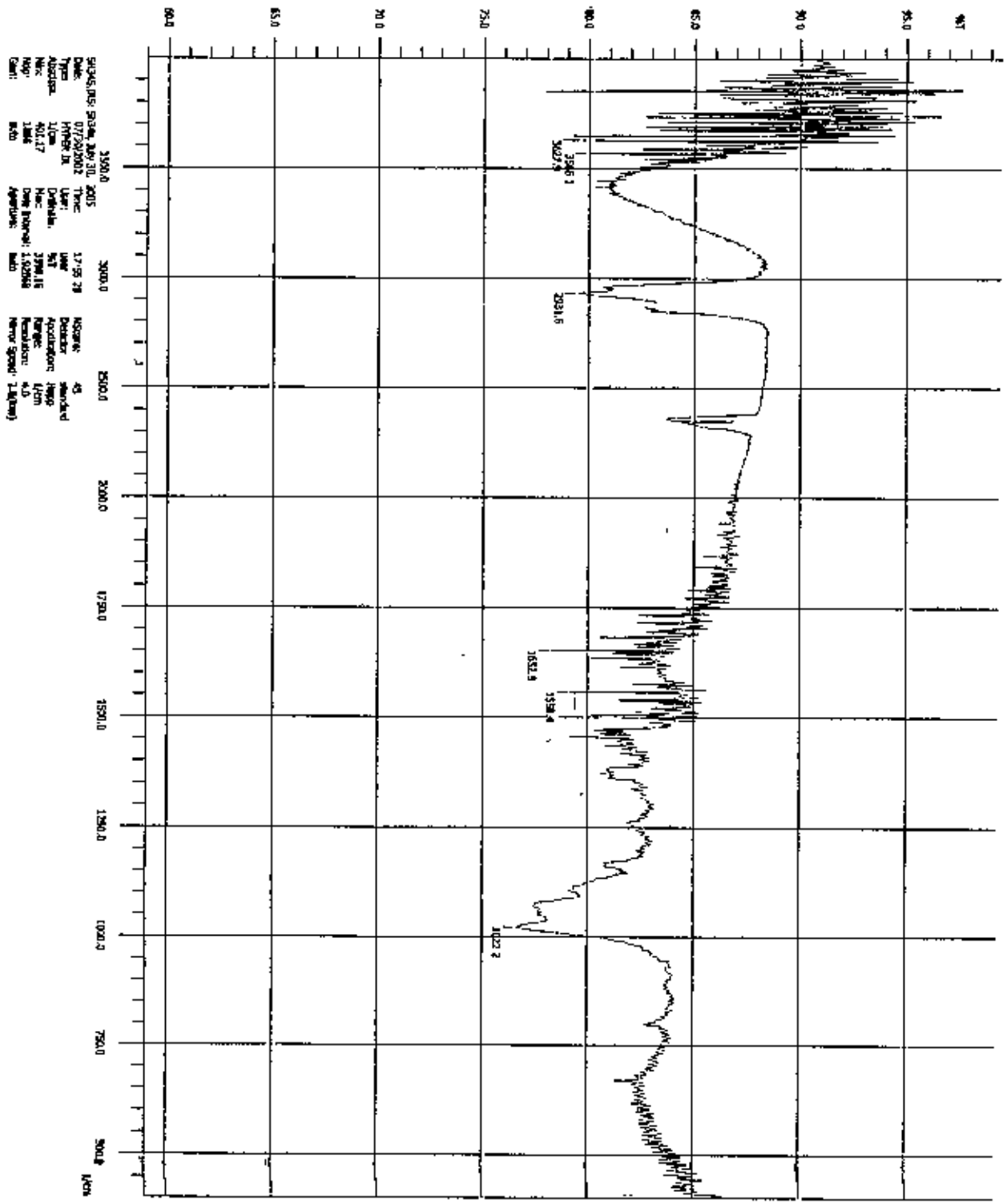

Fig-32: IR spectrum of compound IV

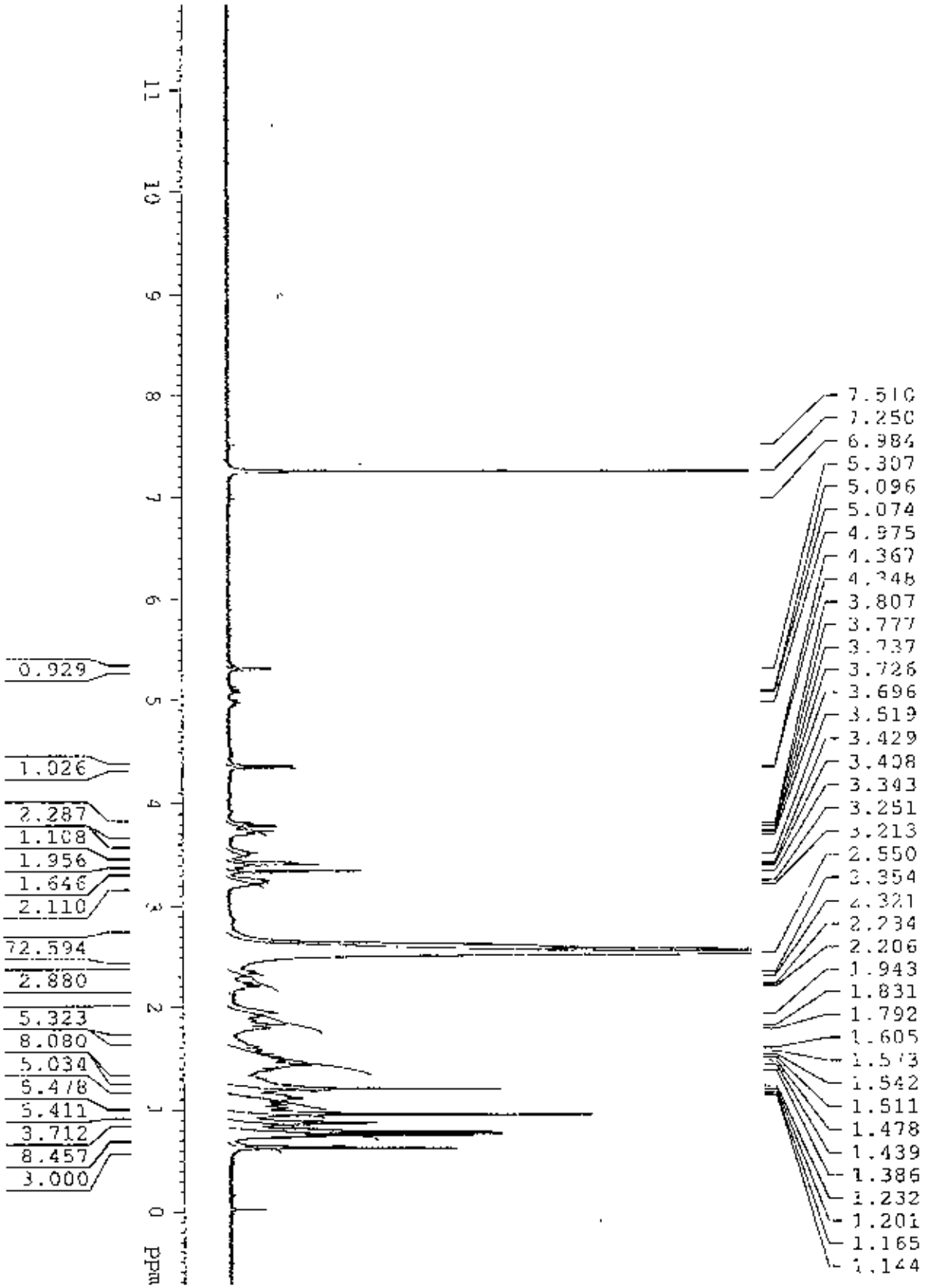

Fig-33: ^{1}H-NMR spectrum of compound IV

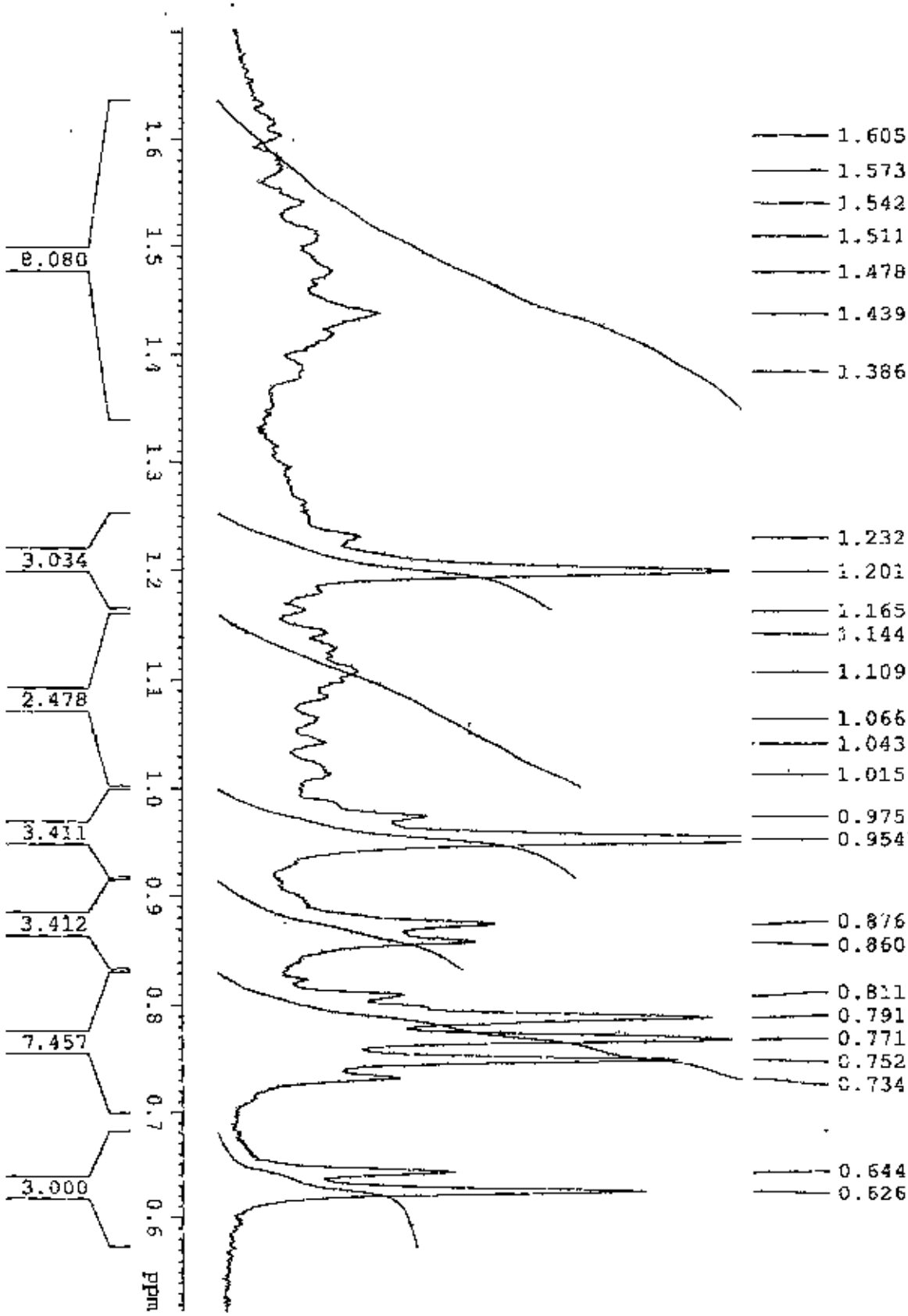

Fig-34: Expansion of ^{1}H-NMR spectrum of compound IV

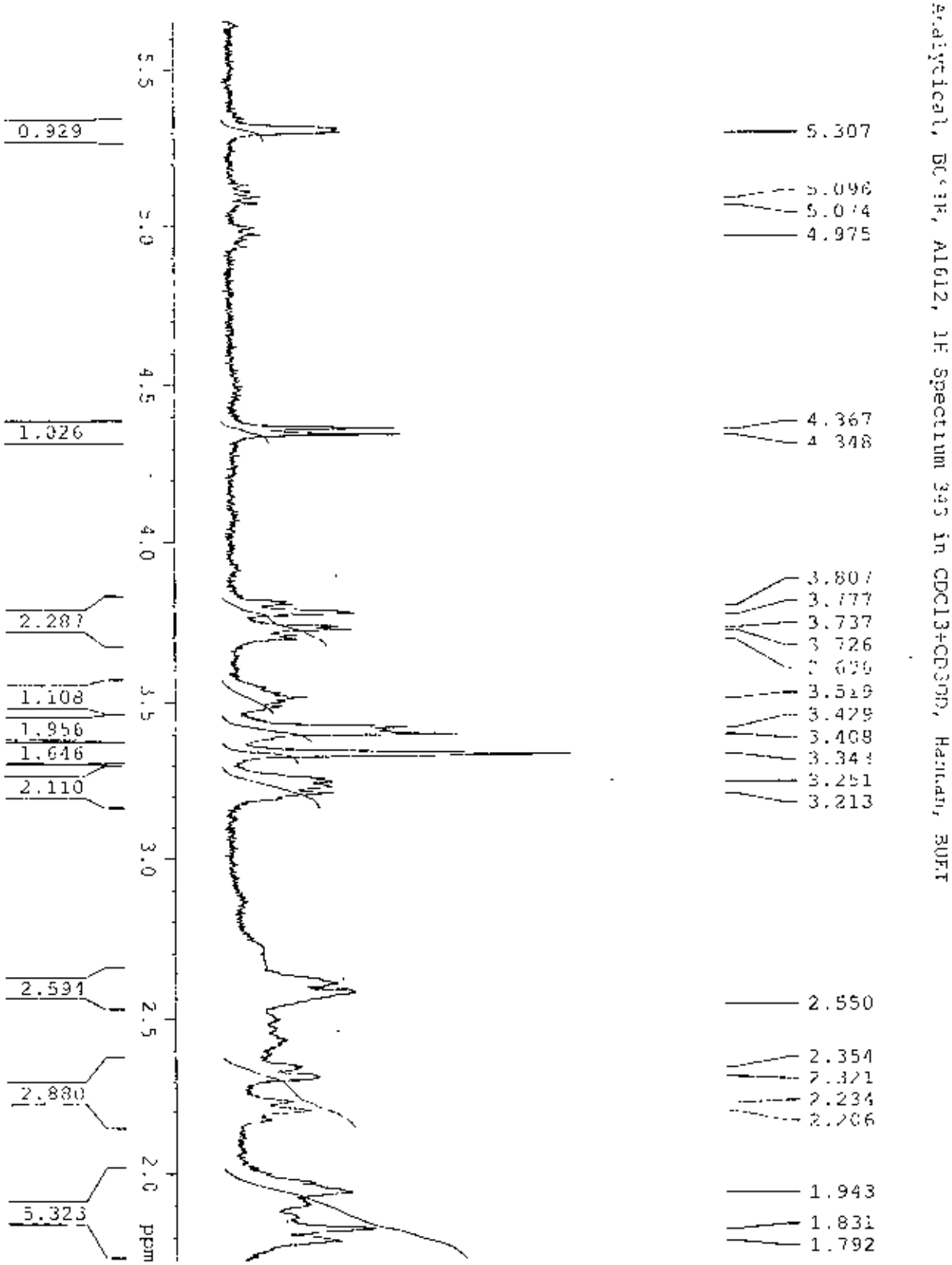

Fig-35: Expansion of ^{1}H-NMR spectrum of compound IV

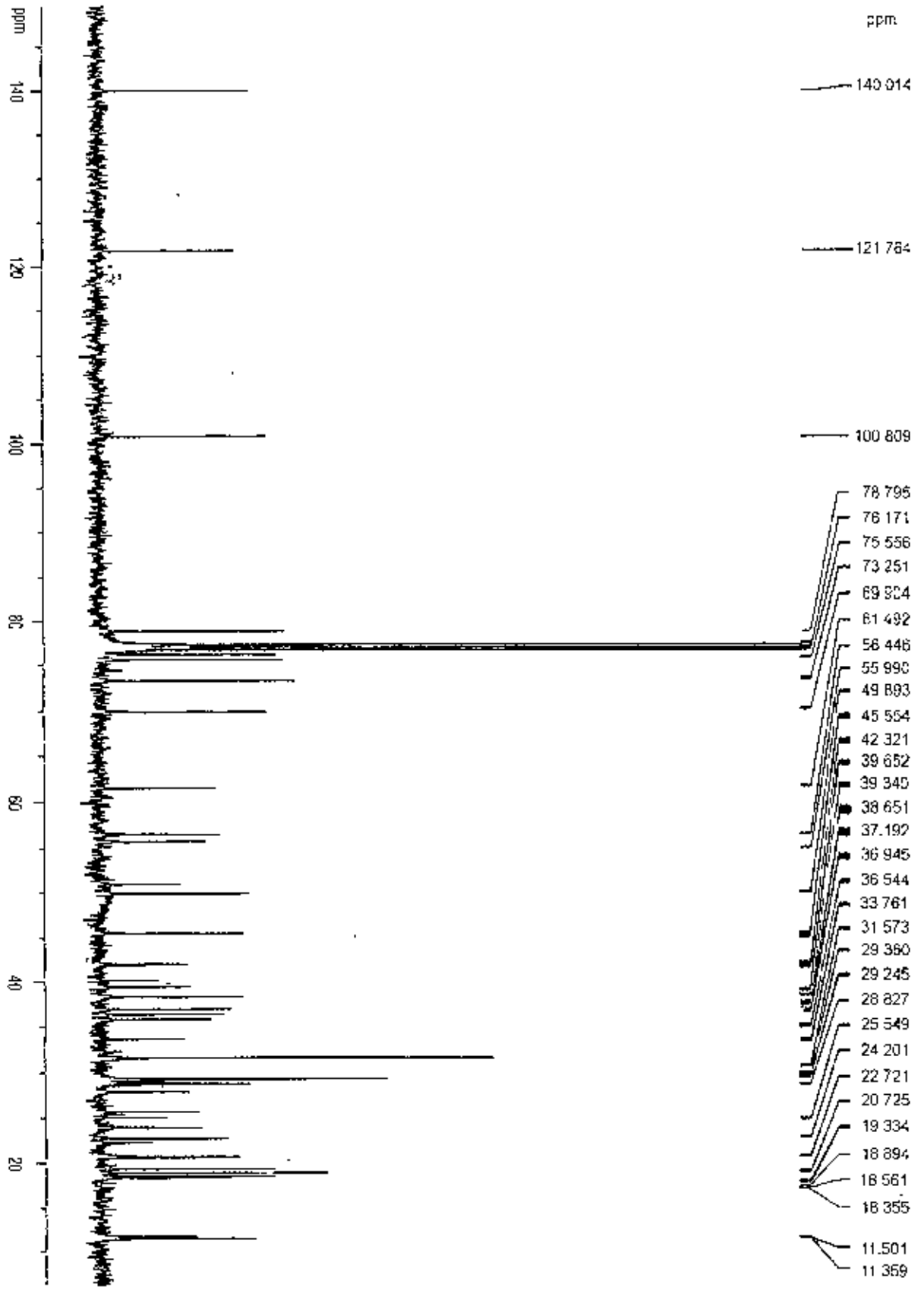

Fig-36: ^{13}C-NMR spectrum of compound IV

3.13 Conclusion:

Dillenia pentagyna is a local plant. During the present investigation four compounds were isolated from the plant extract. Steroidal compound namely β-sitosterol and stigmasterol were isolated from the DCM extract and β-sitosterol glucoside was isolated from the I-Butanol extract. Two other compounds Betulinic acid and lupeol acetate related to pentacyclic triterpenes of lupane series were isolated from the EtOAc extract.

Betulinic acid and β-sitosterol glucoside were bioactive compounds. Betulinic acid is a chemopreventive agent where as β-sitosterol glucoside was found to show hypoglycaemic activity[58-59]

Although the compounds are known compounds but they are first time isolated from the leves of *Dillenia pentagyna.*

The other plants of Dillenia genus are used as a traditional medicine. Extensive chemical works have been done. Literature showed that betuline and betulinic acid are common constituents of this genus plant. But *Dillenia pentagyna* is not recognised as medicinal plant in most literature

So we can justify that the plant *D pentagyna* can be used as a medicinal plant. Since it contains the bioactive compounds[60].

CHAPTER 4

4.0 SUMMARY

Dillenia pentagyna of family Dilleniacea is a local medicinal plant and widely distributed throughout Bangladesh. Its local name is Hargeza. The plant is said to be used in sores, carbuncle, neuralgia, pleur and pneum.

A collaborative research program was done by National Cancer Institute (NCI) Maryland. USA and Department of Chemistry University of Dhaka on the Biological activity of some of the Bangladeshi plant; *Dillenia pentagyna* is one of them.

From the literature survey it is evident that a number of compounds have been isolated from the bark of *Dillenia pentagyna* but the leaves were not studied in details. Therefore the present program is designed to study the leaves of *Dillenia pentagyna*. In this program chemical studies was carried out to isolate pure compounds from the plant extracts and was characterized by chemical and spectroscopic methods.

The leaves were collected from the forest of Modhupur, Tangail. Leaves were cut into small pieces, air -dried, dried in an oven at 40°C and powdered for the present work.

The dried and powdered leaf of Dillenia Pentagyna (1.9 kg) was extracted with a mixture of DCM:MeOH (1:1). The combined extract was filtered of evaporated to dryness and finally dried by high vacuum in a freeze-dryer to give 69.39g (3.65 %) of solid material.

DCM:MeOH extract (69.39g) of leaves was suspended in water (0.5L) and was partitioned with dichloromethane (1Lx3) at room temperature using a separating funnel. After partition two parts were obtained aqueous and dichloromethane part. The dichloromethane part was evaporated to dryness (33.68g) and was further partitioned (Scheme-2) with aq. 90% MeOH : Hexane (1:1). Two parts were obtained, Hexane and

(Scheme-2) with aq. 90% MeOH : Hexane (1:1). Two parts were obtained, Hexane and aq. 90% MeOH part. Both the parts were evaporated to dryness and the solid mass was 10.05g and 19.16g respectively. The aqueous suspension was treated with ethyl acetate (0.5L x 3). The ethyl acetate soluble layer was separated and evaporated to dryness (2.12g). The remaining aqueous part was further partitioned with 1-butanol (0.75Lx3). The 1-butanol soluble part was separated and evaporated to dryness (3.5g)

The aq. 90% MeOH extract (19.16g) was fractionated by silica gel column chromatography and eighy different fractions (DP_1-DP_8) were obtained .Fraction D_6P_3 gave single spot and comply with the positive test of steroid.

The structure of compound I was elucidated from its physical characteristics and various spectroscopic data including UV, IR, ^{1}HNMR AND ^{13}CNMR spectroscopic analysis.

From the spectral data it was found that the compound I was a mixture of two steroids The ^{13}CNMR spectral data were compared with the reported ^{13}CNMR spectral data of similar steroids. From the spectroscopic analysis and comparison with the reported value it was confirmed that the isolated compoundI was actually a mixture of β-sitosterol and stigmasterol.

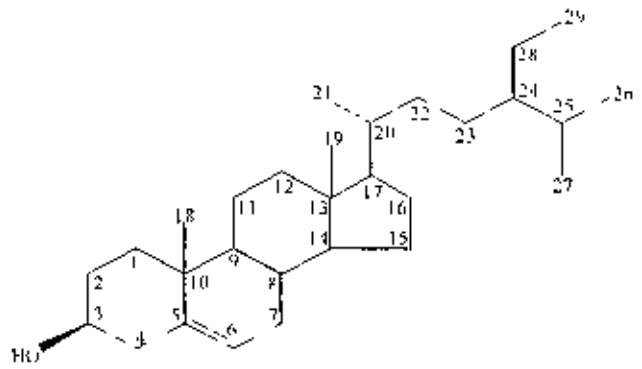

Fig: Structure of β-Sitosterol

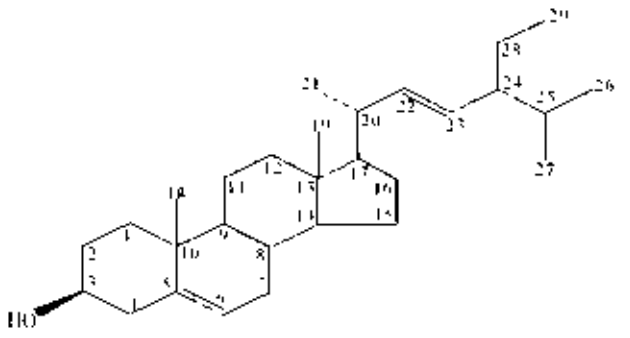

Fig 12(b): Structure of Stigmasterol

Although β-Sitosterol and stigmasterol are known compounds but these are first time isolated from this plant.

The EtOAc extract was fractionated by using dichloromethane followed by methanol. Fraction D_1P_2 gave round spot. It was washed with n-Hexane and crystallized It gave violet colour with vanillin-sulphuric acid reagent indicating that compound II might be a triterpene.

The structure of compound II was elucidated from its physical characteristics and various spectroscopic data including UV, IR, ^{1}H-NMR and ^{13}C-NMR spectroscopic analysis.

The ^{13}C-NMR spectral data were compared with the reported ^{13}C-NMR spectral data of similar compound. From the spectroscopic analysis and comparison with the reported value it was confirmed that the isolated compound II was actually Betulinic acid.

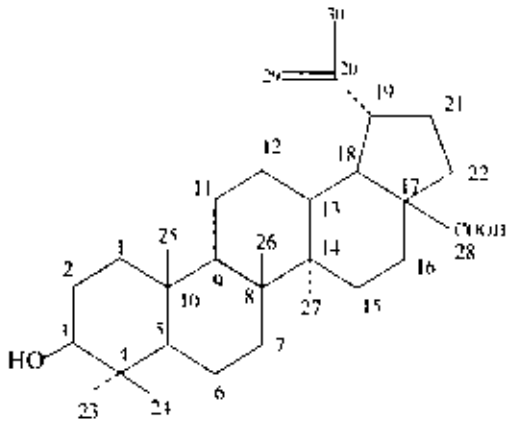

Fig 22: Structure of Betulinic acid

The fraction D_1P_4 was further fractionated by ethyl acetate followed by methanol. The eluted samples gave five different fractions. Fraction D_2P_3 gave round spot. It was further washed with n-Hexane for several times to remove associated coloring materials. The residue was crystallized with ethyl acetate-methanol mixture. The crystals were dried and compound III was obtained.

The structure of compound III was elucidated from its physical characteristics and various spectroscopic data including UV, IR. ^{1}H-NMR and ^{13}C-NMR spectroscopic analysis

The ^{1}H-NMR and ^{13}C-NMR spectral data were compared with the reported ^{1}H-NMR and ^{13}C-NMR spectral data of similar compound. From the spectroscopic analysis and comparison with the reported value it was confirmed that the isolated compound III was actually Lupeol acetate.

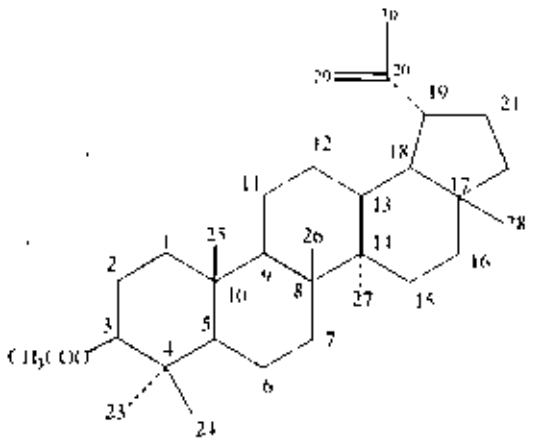

Fig 30: Structure of Lupeol acetate.

Although Lupeol acetate is a known compound but it is first time isolated from the leaves of this plant.

The 1-Butanol extract was fractionated by a Sephadex LH-20 gel column Among all he fraction D_3P_6 gave a spot with small tailing. It was then further fractionated by a silica gel column. The column was first eluted by dichloromethane and the polarity of the solvent was gradually increased by the addition of methanol. Five different fractions were obtained according to their R_f values on TLC plates. Fraction D_4P_2 gave round spot It was further washed with n-hexane for several times to remove associated coloring materials The residue was white solid crystal. The crystals were dried and compound IV was obtained

The structure of compound IV was elucidated from its physical characteristics and various spectroscopic data including UV, IR, ^{1}H-NMR and ^{13}C-NMR spectroscopic analysis.

The ^{13}C-NMR spectral data were compared with the reported ^{13}C-NMR spectral data of similar compound. From the spectroscopic analysis and comparison with the reported value it was confirmed that the isolated compound IV was actually β-sitosterol glucoside. It was also first time isolated from the leaves of *Dillenia pentagyna*.

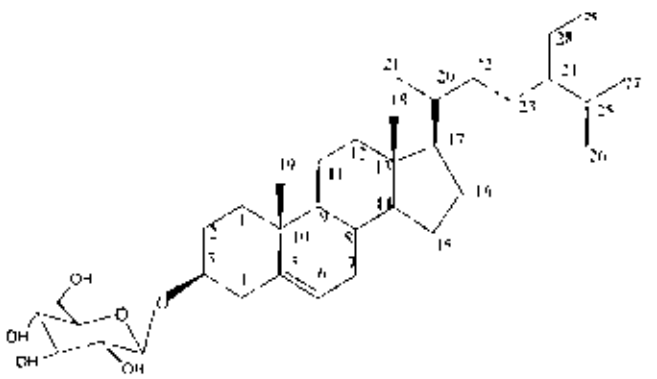

Fig 37: Structure of β-sitosterol glucoside.

Dillenia pentagyna is a local plant. Four compounds were isolated from the plant extract. Steroidal compound namely β-sitosterol and stigmasterol were isolated from the DCM extract and β-sitosterol glucoside was isolated from the 1-Butanol extract. Two other compounds Betulinic acid and lupeol acetate related to pentacyclic triterpenes of lupane series were isolated from the EtOAc extract.

Betulinic acid and β-sitosterol glucoside were bioactive compounds. Betulinic acid is a chemopreventive agent where as β-sitosterol glucoside was found to show hypoglycaemic activity[58-59].

Although the compounds are known compounds but they are first time isolated from the leaves of *Dillenia pentagyna*.

The other plants of Dillenia genus are used as a traditional medicine. Literature showed that betulin and betulinic acid are common constituents of this genus plant. But *Dillenia pentagyna* is not recognised as medicinal plant in most literature.

So we can justify that the plant *D. pentagyna* can be used as a medicinal plant. Since it contains the bioactive compounds[60].

CHAPTER 5

5.0 REFERENCES

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