Phytochemical Studies on some species of the Amaranthaceae Family

M.Phil. Thesis

By

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A thesis submitted to the Department of Chemistry, University of Dhaka, for the Degree of Master of Philosophy in Chemistry

by

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September, 2010

DEDICATED

TO

MY PARENTS & TEACHERS

CERTIFICATE

This is to certify that Mirza Jesmine Begum, an M. Phil. student bearing registration No.: 244 (session: 2001-2002), of the Department of Chemistry, University of Dhaka, has carried out her research work leading to M. Phil degree under our joint supervision at the Organic Research Laboratory of our department. It is to mention that she has done all her research work by herself and she has prepared the thesis entitled **"Phytochemical Studies on some species of the Amaranthaceae family".** The results incorporated in this thesis has not been used or submitted elsewhere for award of any other degree.

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DECLARATION

I do hereby declare that this dissertation entitled "**Phytochemical Studies on some species of the Amaranthaceae family".** Department of Chemistry (Organic Chemistry) University of Dhaka, Dhaka, Bangladesh, in fulfillment of the requirement for the Degree of Master of Philosophy (M. Phil.) is the result of my own investigation.

I further declare that the work embodied in this thesis has not already been submitted in substance for the award of any degree or diploma.

Date: 29-09-2010

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OBJECTIVE

Bangladesh is rich in numerous kinds of plants, herbs and creepers and most of them have medicinal value. However, a very little is known about the chemical composition of the plants. Therefore, studies on the isolation and characterization of the medicinally important compounds from them are very important for the well being of human society. *Amaranthus spinosus* L. is one of these types of medicinally important plant. The bark, leaves and seeds are used by Ayurvedi, Yunani doctors and local kobiraji for remedy of mild internal bleeding, topical for eczema, dysentery, indigestion, gall stones, gall bladder inflammation, genital discharge, coughing up blood etc. Investigation on this species has been carried out by different researchers but more investigations are needed to explore its therapeutic value. With this end in view, this species has been taken into consideration with a keen interest to investigate the phytochemicals.

CHAPTER ONE

INTRODUCTION

1. INTRODUCTION

1.1 BACKGROUND

Plants play an important role[1] in our life. Parts of various plants meet our daily necessities. Three basic needs of our human life, i.e. food, clothing and shelter are primarily obtained from plant kingdom. A major part of the global energy requirements is also supplied by the plants source as fuel. Plants also afford many useful organic chemicals. They supply many hydrocarbons, which are the key starting materials for the production of synthetic dyes, plastic, insecticides (DDT), sulfa and other drugs. The simple compound (CO_2) and water (H_2O) combine in the leaves of plant in the presence of chlorophyll and sunlight to produce glucose and the byproduct oxygen, which are essential for all forms of life. Plants play a vital role in consuming CO_2 from atmosphere and save it from the negative impression of the green house effect. Wood pulp is used for making paper. It is convertible into artificial silks, guncotton, automobile lacquers and plastic. Certain trees, shrubs, flowers and fruits contain organic chemicals of values as plasticizers (camphor, for celluloid), perfumes, flavors and paint gradients (turpentine). Plants produce not only food materials but also other organic compounds such as glycosides, steroids, terpenoids, alkaloids, pigments etc.

The plant kingdom was the drug store[1] for countless centuries. Today the number of plants directly used in medicine has been decreased but they are hidden in many pills, capsules and medicine bottles as active chemicals originally found in the plant kingdom.

In the beginning to the nineteenth century, when modern chemistry and pharmacy began to develop, the original impetus to the study of natural products chemistry utilizing medicinal plants was started to develop. In the recent decades, natural pesticides, antibiotics, vitamins and hormones are the follow up results of the researchers of chemistry of natural products.

Scientists are now working together to find out new drugs from plants for incurable diseases likes diabetes, cancer and AIDS. So, it will not be at all to therapy of the controlled diseased.

Ordinary people do not know why a plant is effective against particular diseases and the same time, they also do not know what are the side effects and toxic ingredients of such plants materials. So, the chemists have a great responsibility to find out the active ingredients, which are effective against various diseases and also to indentify the toxic ingredients if any.

1.2 MEDICINAL IMPORTANCE OF PLANT MATERIALS

Medicinal plants[2] possess therapeutic properties or exert beneficial pharmacological effects on the animal body. A medicinal plant contains substances that can be used for therapeutic agents. Natural products and their derivatives analogs still represent till over 50% of all prescribed drugs. Plants have defense mechanism against animal perdition, fungal attack, microbes and viruses. According to one estimation only 5% of the 5,00,000 species of plants have been screened for biological activity.

Uses of folk or traditional medicine represent the way of shortcut discovery of modern medicine. An inventory of medicinal plants compiled by WHO on the basis of literature from 91 countries including the classical text on Ayurvedic and Unani medicine list 21000 species of "medicinal plants". According to WHO, around 80% of the world's 5.76 billion populations in the developing world rely on herbal remedies for their basic health care need.

The use of medicinal plants as a source for relief from illness can be traced back over five millennia from written documents of the early civilizations in China, India and near east but it is doubtless an art as old as mankind. Even today, plants are the almost exclusive source of drugs for the majority of the world's population. In industrialized countries, medicinal plant research has had its ups and downs during the last decades. Plants[2] will continue to be extremely important as source of new drugs as evidenced by recent approvals in the United States of several new plant derived drugs based on the secondary metabolites of plants. For example, in the treatment of refractory ovarian cancer, new drug has recently been approved in the United States from taxol, an anti cancer taxane diterpenoid derived from the relativity scarce pacific western Yew tree, *Taxus brevifolia* Nutt. A relatively new semi-synthetic antineoplastic agent based on podophylotoxin is etopiside a constituent of the Mayapple *Podophyllum petatum,* which is useful in the chemotherapeutic treatment of refractory testicular carcinomas,

small cell lung carcinomas, non-Hodgkin's lymphoma and non lymphocytic leukemia. The list of modern medicine derived from medicinal plants[3,4] is very long now.

1.3 THE AMARANTHACEAE FAMILY

The Amaranthaceae family[5] is based in the generic name *Amaranthus*. The *Amaranthus* is presumed to be derived from the Latin Amaranth meaning an imaginary unfading flower. The potherbs family Amaranthaceae is a large family comprising of about 65 genera and more than 1000 species distributed throughout the world, but most abundant in tropical regions and especially tropical America and Africa.

In the famous book 'Bengal plants' listed altogether 26 species and 4 varieties under 12 genera. Most of them occur in open places, west places, roadsides, fields, low lands, swamps and subcontinent is given for Bengal by Prain[6], specific localities are rarely cited. Datta and Mitra published[5] a general record of the caption "Common plants in and around Dacca" in which fourteen (14) species and one variety of Amaranthaceae are listed under nine (9) genera.

1.4 SPECIES OF AMARANTHACEAE AVAILABLE IN BANGLADESH

The plant of this species[6] grows abundantly in Bangladesh. Some of the species are listed below.

TABLE-1.1: SPECIES OF AMARANTHACEAE AVAILABLE IN BANGLADESH.

Scientific Name	Native Name
Amaranthus spinosus L.	Katanotey, Kanta-nutia, Kanta-miris
Amaranthus blitium L.	Natiya sag
Amaranthus dubius Mart.	Dengue, Hal-sha, Kata Notey, Gobra
Amaranthus gangeticus L.	Lal Sag
Amaranthus lividus L.	Data Sag
Amaranthus mangostanus L.	Gobua notey
Amaranthus tenuifolius Wild	Genti-nuli, Gelchumli
Amaranthus virdis L.	Notey, Noaty Sag, Maris Sag

1.5 ECONOMIC IMPORTANCE OF AMARANTHACEAE:

Economically this family is very important[7] and can be described under the following heading:

Fodder: *Alternanthera parnychioides, Alternanthera puingens, Alternanthera sessilis* and some species of are used as fodder.

Food: *Alternanthera parnychioides, Alternanthera pungens, Alternanthera sessillis, Amaranthus bubius, Amaranthus gengiticus are highly esteemed green vegetables.* Leaves of *Amaranthus graecijans, Amaranthus spinosus, Amaranthus tenufolies, Amaranthus virdis* are sometimes eaten as spinach. *Celosia argetia* is used as a pat herb in times of scarcity; leaves furnish an inferior vegetable. Young Cooked sprouts of Deeringia amaranthoids are eaten with rice.

Soil binder: The gregarious matted growth *Altenanthera bettzixkiana, Alternanthera ficoides, Alternanthera paronchiodes, Alternanthera pungens* are sometimes used for protecting soils from rain-wash.

Purifier: *Alternanthera sissilis* is used as a climate purifier.

As garden plants: *Alternanthera bettzixkiana, Alternanthera ficoides, Alternanthera gagiticus, Celosia argentia, Gomphyra globosa* are planted as ornamental plants in the garden.

1.6 MEDICINAL IMPORTANCE OF AMATANTHUACEOUS PLANTS:

Most of the plants of Amarnthaceae are used as medicine[8] in various diseases such as -

Dropsy: *Achranthes aspera, Celosia argentea* are used is dropsy.

Dysentery: *Achranthes aspera, Amaranthus gangiticus, Celosia argentea* are used in dysentery.

Piles: *Achranthes aspera, Amaranthus spinosus, Celosia argentea* are used in piles.

Cough:*Aerva lantana, Celosia cristata* are valued for cough.

Bronchitis: *Amaranthus spinosus* is used in bronchitis.

Headache: *Aerva lanata* is used in headache.

Menstruation: *Aerva sanguinolenta, Amaranthus blitum, Alternanthera gangeticus* are used against irregular or painful menstruation.

Leucorrhoea: *Amaranthus spinosus* is used in leucorrhoea.

Rheumatism: *Achyranthes aspera* is used in rheumatoid troubles.

Demulcent: *Aerva lanata, Amaranthus spinosus, Alternanthera gangeticus, Celosia cristata* are used in diuretic.

Stomachic: *Amaranthus blitum, Amaranthus spinosus, Celosia cristata* are used as stomachic.

Biliousness: *Aerva lanata, Amaranthus gangeticus, Alternanthera spinosus, Celosia cristata, Digera muriceta* are useful in biliousness.

Liver complaints: *Amaranthus gangeticus, Celosia argentea* are used in liver complaints.

Ulcer and sores: *Amaranthus gangeticus* is used as wash for ulcer and sores.

Blood diseases: *Achyranthus aspera, Aerva lanata, Alternanthera sanguinnlenta, Amaranthus spinosus* are used is blood diseases.

Leprosy: *Amaranthus spinosus* is used in leprosy.

Laxative: *Amaranthus spinosus, Digera muricetata* are used in Laxative.

Suppuration: *Amaranthus spinosus* are used as remedy against sudden swellings.

Rate-bite: *Amaranthus spinosus* is used in rate-bite.

Febrifuge: *Alternanthera sessilis, Amaranthus spinosus, Celosia argentea* are used as febrifuge.

1.7 BOTANICAL CHARACTESISTICS OF THE FAMILY AMARANTHACEAE[9]

Habits: It may be annul, herb, perennial woody herb, shrub. Herb may be erect, diffuse, ascending, prastrate, stem usually solid but rarely fistular.

Spine: Most of the species of Amaranthaceae are unarmed, Using spine character, *Amaranthus spinosus* can be differentiated from all other species of the family occurring in Bangladesh.

Leaves: Most of the species has prtiolate leaves but some of the species has sessile leaves (*Alternanthera sessilis).* Leaves are entire (*Alternanthera ficooides),* obscurely dentate (*Achyranthus aspera*). They may linear, ovate, elliptic, spathulate, rhomboid etc.

Hairs: Stems hairy (*Achyranthus, Aerva, Altenanthera)* or glabrous (*Amaranthus).* Hairs may be pilose (stem and leaf of *Gomphera celosiodes),* stellate, hirsute, wolly, celoate etc.

Flowers: Flowers usually bisexual, sometimes unisexual (*Amaranthus).* It may be solitary (*Achyranthes)* or clustered (*Amaranthus).*

Inflorescence: Inflorescence may be simple spike (*Amaranthus aspera),* compound spike (*Amaranthus spinosus),* coescomb like spike (*Celoia cristata),* raceme

(*Deeringice, Amaranthus)*, cluster (*Alternanathera)* or head (*Gompherena globosa).* They may be auxiliary (*Aerva lanata)* or terminal and auxiliary (*Celosia).*

Bracteals: Bracteals usually shorter or equal, rarely greatly enlarged than bract (*Comphyrena).* It may be ovate (*Amaranthus),* lanceolate (some of the species of *Altenanthera)* or navicular (*Gomherena globosa).* It may be spiny (*Achyranthes)* or not (*Cyathula protata).*

Perianth: Perianth-segments usually imbicate. It may be lanceolate (*Celosia)* ovate-lanceolate (*Achyranthes).*

Fruit: Fruit may be utricle (*Amaranthus),* (*Deeringia).* It may be membranous [Aerva or corky (*Amaranthera)].* It may be dehiscent irregularly (Aerva).

Seed: Seed may be oblong (*Achyranthes),* neniform (*Aerva),* centicular (*Amaranthus),* rhombic (*Digera).*

1.8 GENERAL DESCRIPTION OF *Amaranthus spinosus* L.

Common name(s) [10,11]:

Bangla:	Kata Notey
English:	Edlebur, Needle Burr, Spiny Amaranth, Thorny Pigweed.
French:	Epinard Cochon, Epinard Malabre.
Spanish:	Bledo espinosos, Espinaca de Malabar

Fig 1.1: The plant *Amaranthus spinosus* L. of: Amaranthaceae Family

1.9 Habit: herb

Description: "Monoecious annual"[10,11] herbs' steams sometimes tinged with red, erect, sometimes ascending, 4-15 dm long, usually branched, striate, glabrous or pubescent with multi-cellular hairs, especially above, most leaf axils with a pair of divergent spines up to 2.5 cm long. Leaves ovate to rhombic-ovate, elliptic, lanceolate-oblong or lanceolate, blades 1-12 cm, 0.89-6 cm wide, glabrous, lower surface occasionally sparsely pilose, especially along the veins, petioles 1-9 cm long. Flowers green, in auxiliary clusters in the lover part of the plant and is unbranched or branched spikes in the supper part, the lower clusters entirely pistillate as are the lower flowers of the spikes, the upper flowers in the spikes staminate, bracts and bracteoles delate-ovate, membranous, tipped with a pale or reddish awn; sepals 5, those of staminate flowers broadly lanceolate or lanceolate-oblong, 1.5- 2.5 mm long, apes obtuse or acute, mucronulate ; stigmas (2) 3. Fruit ovoit ovoid, with a short inflated neck below the style base, ca. 1.5 mm long, regularly or irregularly circumscissile, rarely

indehiscent, the upper part rugulose below the neck. Seeds black, shiny, compressed, 0.8-1 mm long, inconspicuously reticulate.

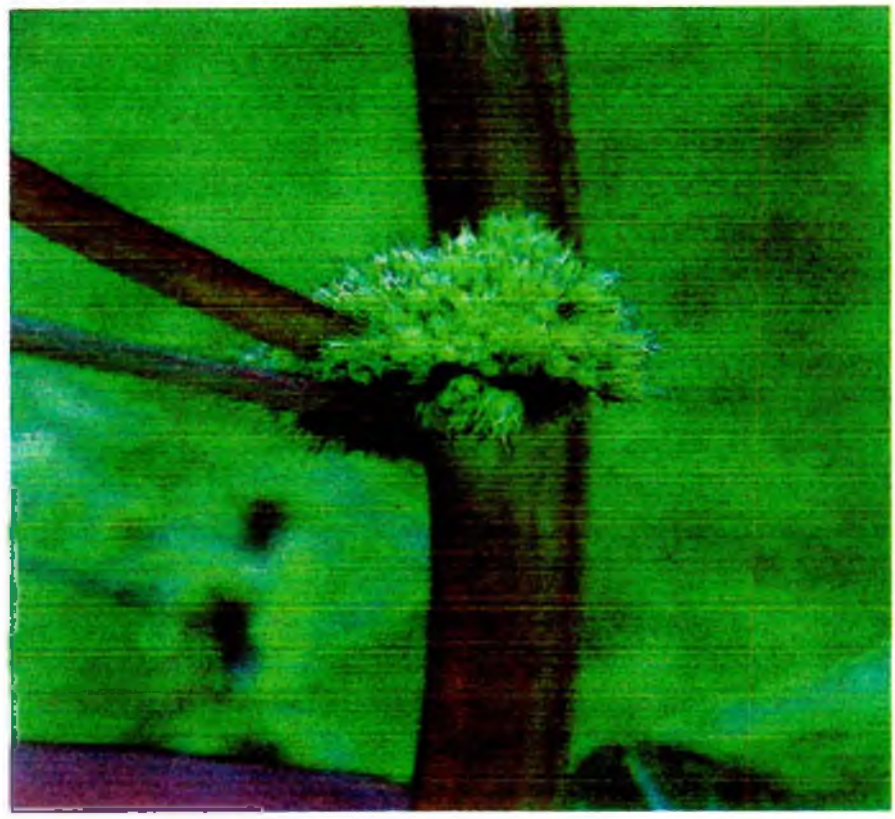


Fig 1.2: The flower of *Amaranthus spinosus* L.

1.10 MEDICINAL IMPORTANCE OF THE PLANT *Amaranthus spinosus* L.

Pharmaceutical Name[12,13]

Functions: Astringent, diuretic, laxative, anti-inflammatory, hemostatic, clears heat and poison, improves digestion, stops bleeding.

USES: [12,13]

- Cardiovascular: Mild internal bleeding [China, India]
- Desmatological: Topical for eczema [China, Nepal]
- Digestive: Enterities, dysentery [China] Indigestion [India] Mild diarrhea [USA]
- Hepatic: Gall bladder inflammation, gall stones [China]
- Infection: Topical for boils, abscesses [China, Nepal] Fever [China]
- Reproductive: Excess menstrual bleeding [Cherokee, India, Nepal] Gonorrhea, genital discharge [India, Nepal]
- Respiratory: Coughing up blood ([India]
- Part used: Root and steam.

Clinical combinations: With eclipta prostrate for bleeding [China] Steeped with lavender or chamomile for colic or vomiting in children.

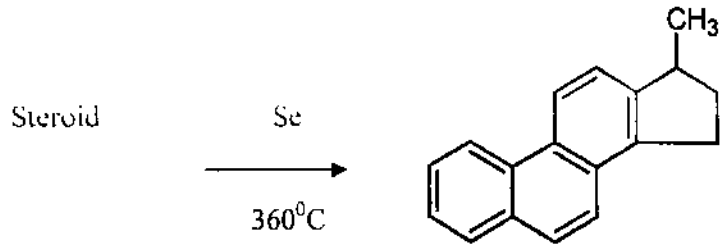
Caution: Not in pregnancy.

Toxicity: Mildly toxic overdo sage causes dizziness, nausea and vomiting.

Preparation: It is used after drying.

1.11 STEROIDS

The steroids form a group of structurally related compounds which are widely distributed in animal and plants. Included in the steroids are sterols, vitamin D, some carcinogenic hydrocarbons, certain sapogenin etc. The structures of the steroids based on the 1,2-cyclopentanoperhydrophenanthrene skeleton. All the steroids give, among other products, Diels' hydrocarbon on dehydrogenation with selenium at 360°C[14]. Most eukaryotic cells contain and synthesize sterols. Whereas cholesterol is the unique sterol of vertebrates and ergosterol the major sterol of most fungi and some unicellular algae, most higher plants contain a complex mixture in which cholesterol is a minor component and 24-ethyl sterols (sitosterol and stigmasterol) account for more than 60% and 24-methyl sterols account for less than 40%[15].



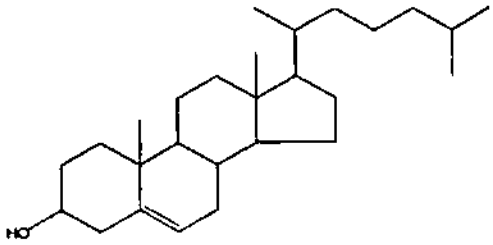
Diel's hydrocarbon

Steroids contain four rings fused together. Among the four rings three are six membered and other is five membered.

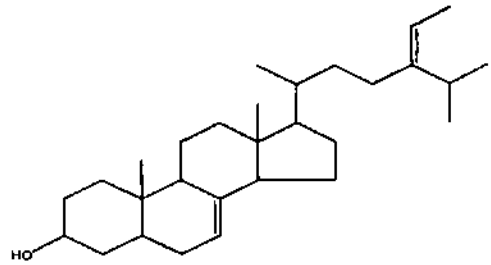
The precursor of steroids in biological system is squalene, a triterpene which was originally produced by joining of six isopentenyl pyrophosphate. Many hormones, bile acids and vitamins have this steroidal structure. Usual birth control pill from women contains steroidal hormones. Steroids are extractible by lipophilic solvents like chloroform, petroleum ether etc.

1.12 BIOSYNTHESIS OF STEROIDS

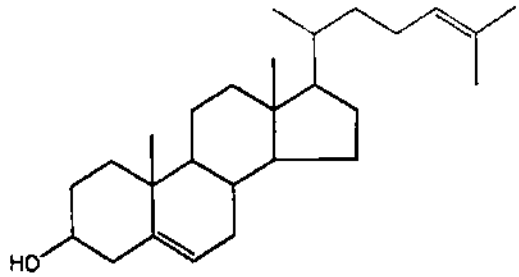
Steroids[15,16] are not triterpenes as they possess C_{27}-C_{29} skeletons; rather, although both of them are derived from the same C_{30} precursor squalene. This squalene is derived from two farnesyl pyrophosphate units, upon joining each other in the unusual 'head to head' fashion.



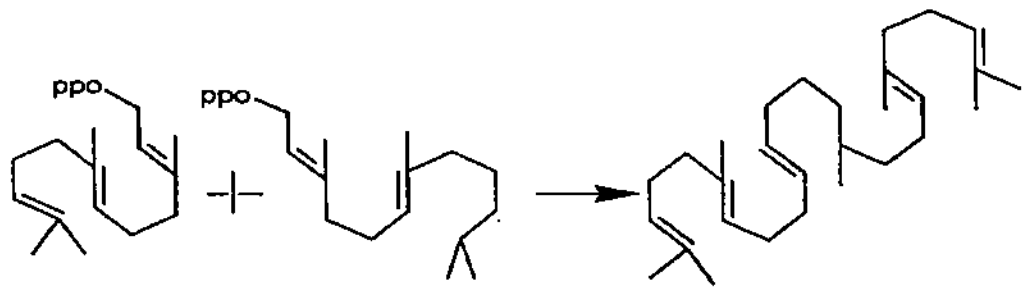
Cholesterol (Cholest-5-en-3β-ol)



Avenasterol



Cholesterol (5,24-cholestadienol)



Squalene

Cyclisation of squalene-2,3-epoxide in a chair-boat-chair-boat conformation and by a subsequent sequence of rearrangement leads to lanosterol, cycloartenol etc. Desmosterol is formed from lanosterol by a sequence of medication reactions. β-sitosterol and stigmasterol are formed by the addition of extra carbon atoms to the side chain of desmosterol in plants.

1.13 LITERATURE SURVEY ON THE PHYTOCHEMICAL INVESTIGATIONS AND MICROBIAL ACIVETIES ON *Amaranthus spinosus.*

Considering the importance of different species as a medicinal plant, extensive chemical works have been carried out on the leaves, a barks and seeds by different researches. Brief reviews of the previous works are given below:

The antifungal activity of aqueous and organic solvent extracts of four weedy plants species viz. *Tagetes minuta, Lippia, Javanica, Amaranthus Spinosus and Vigna unguiculate* has been investigated[17].

Comparative study in vitro anthelmintic activity of three plants of the Amaranthaceae family, *Amaranthus spinosus*, *Amaranthus caudatus* and *Amaranthus viridis* has been carried out[18] .

Hepatoprotective activity of *Amaranthus spinosus* on animals was tested[19].

In vivo antimalarial activities of extracts from *Amaranthus spinosus* was carried out[20].

Anti-inflammatory properties of *Amaranthus spinosus* leaf extract was studied[21].

In 2003, betacyanins and phenolic compounds form *Amaranthus spinosus* have been isolated and it was found that the extracts of stems of *Amaranthus spinosus* contained hydroxycinnamates, quercetin and kaemferol glycosides[22].

Nutritional and anti nutritional components of *Amaranthus spinosus* were analyzed, recently. The evaluated parameters were protein, carbohydrate, ascorbic acid, phenol, flavanoid, moisture and oxalate[23].

A survey of wild, green, leafy vegetables (e.g. *Amaranthus spinosus)* and their potential in combating micronutrient deficiencies in rural population was done[24].

Total carotenoid and β-carotenes contents of forest green leafy vegetables (e.g. *Amaranthus spinosus*) consumed by tribal's of South India was studied[25].

Bioavailability of iron from *Amaranthus spinosus* supplemented maize diet has been studied and the study revealed that iron bioavailability was maximum when diet containing 50% maize and 50% fresh amaranth leaves was fed to the albino rats[26].

Composition of the seeds of *Amaranthus spinosus* has been studied in 1998[27].

Vitro availability of iron, in various green leafy vegetables (e.g. *Amaranthus spinosus)* has been studied and vitro availability of iron from this plant was calculated from ionizable ions in 1988[28].

Besides the above mentioned works, it has also been found that leaves and stems contain n-alkanes, hentriacontane, octacosanoate, sterols including alpha-spinasterol, saponins, fatty acids, free alcohols and proteins[29-31]. It also contains phosphorus, iron, nicotinic acid, ascorbic acid and protein[32]. Roots contain alpha-spinasterol, octacosanoate and a number of saponins [31].

A new coumaroyl flavone glycoside was isolated from the n-butanol fraction of the methanolic extract of the whole plant of *Amaranthus spinosus*[33].

CHAPTER TWO

METHODOLOGY & EXPERIMENTAL

2. METHODOLOGY

2.1 GENERAL METHODS

2.1.1 DISTILATION OF THE SOLVENTS

The commercial grade solvents (petrol, ethyl acetate, chloroform and methanol) were distilled. Petroleum ether (b.p 40-60)^{0}C was obtained by distilling petrol. Distilled solvents were used through out the investigation.

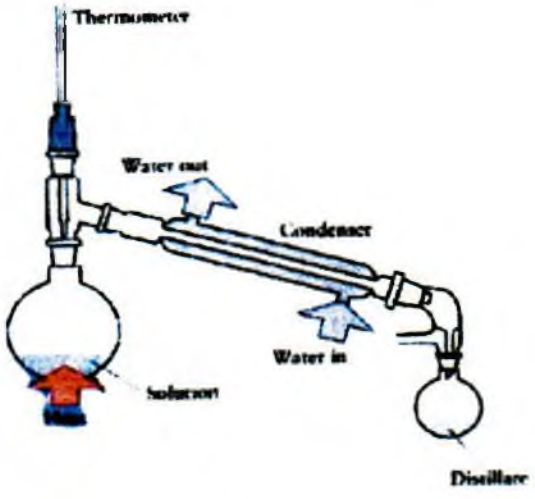


Fig 2.1: Distillation pump.

2.1.2 EVAPORATION

All evaporation were carried out under reduced pressure using rotary vacuum evaporator at bath temperature not exceeding 40^{0}C

2.1.3 PREPARATION OF THE REAGENTS

Fig 2.2: Vanillin-Sulphuric acid spray

2.1.3: VANILLIN-SULPHURIC ACID REAGENT

Sulphuric acid (400 ml) and absolute alcohol (100 ml) were mixed in a beaker (kept in ice bath). Vanillin (0.025 g) was added to the mixture of alcohol and sulfuric acid, cooled and used for spraying the TLC plates.

2.1.4 CHROMATOGRAPHIC TECHNIQUES

Two types of chromatographic techniques were used such as thin layer chromatography (TLC) and vacuum liquid chromatography (VLC).

2.1.4.1 THIN LAYER CHROMATOGRAPHY (TLC)

Two types of TLC plates were used through out the experiments;

i) Precoated TLC plates: 0.2 mm thin coatings of silica gel on glass plates or aluminums sheets were used.

ii) Manually prepared silica gel coated glass was used.

2.1.4.2 PREPARATION OF PLATES

Thin layer chromatographic plates were prepared by spreading a film of an aqueous slurry (gel: water = 1:2 w/v) of silica gel G-60 PF_{254} (E, Merck 7731) over the entire surface of the glass plates (6cm × 12cm) by means of spreader. The thickness of the silica gel layer was 0.2 mm. The plate was air and activated by heating at 110^0C for 1 hour followed by cooling at room temperature for few hours.

2.1.4.3 SAMPLE APPLICATION (SPOTING THE PLATES)

The TLC plates were spotted with a small amount of the crude extract by using a narrow glass capillary. The capillary was wash with either acetone or ethanol before each sample applied.

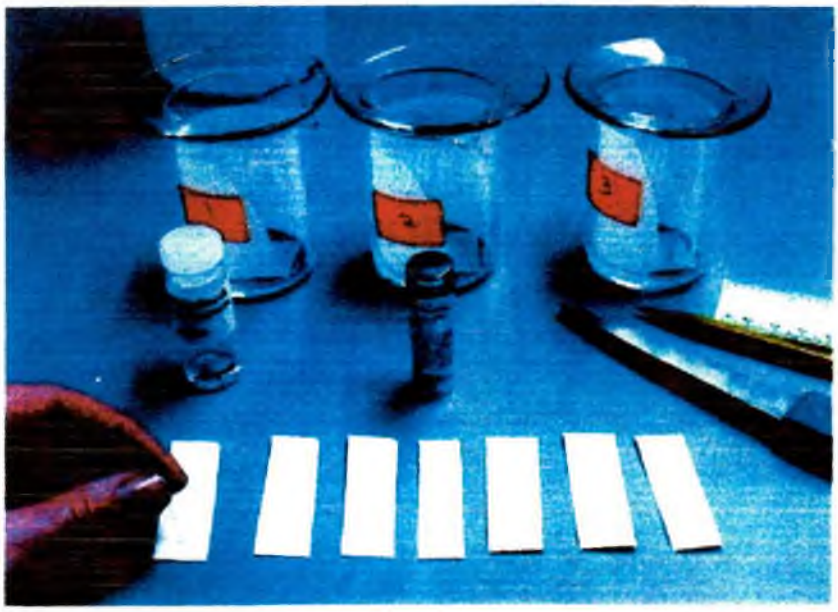


Fig 2.3: process of spotting

2.1.4.4 SOLVENT SYSTEM

The solvents of different polarity used for TLC are given below:

i) Petroleum ether: Dichloromethane (in different ratio)

ii) Petroleum ether: Ethyl acetate (in different ratio)

iii) Dichloromethane: Methanol (in different ratio)

iv) Ethyl acetate: Methanol (in different ratio)

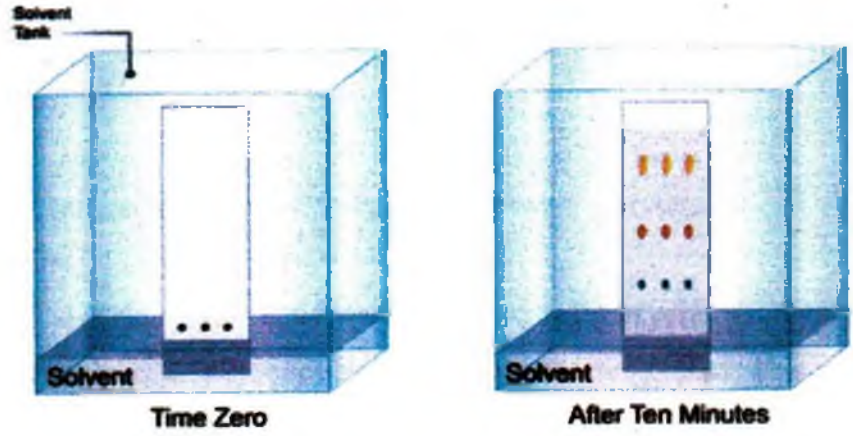


Fig 2.4: Developing of TLC plate

2.1.4.5 PREPERATION OF TLC TANKS

The ascending technique in glass jars or tanks were used to develop TLC plates. A suitable solvent system was poured into glass jar or tank in a sufficient amount. The tank was then covered with a lid and kept for a certain period allowing it to achieve saturation. A filter paper was usually introduced into tank to promote the saturation process. The solvent level at the bottom of the tank must not be above the line of the spot where the sample solution was applied to the plate. As the solvent rises, the plate becomes moistened. When the solvent front moves almost near the end of the plate, the plate was then taken out and dried. The solvent front was not allowed to travel beyond the end of the silica-coated surface.

2.1.4.6 DETECTION OF SPOTS

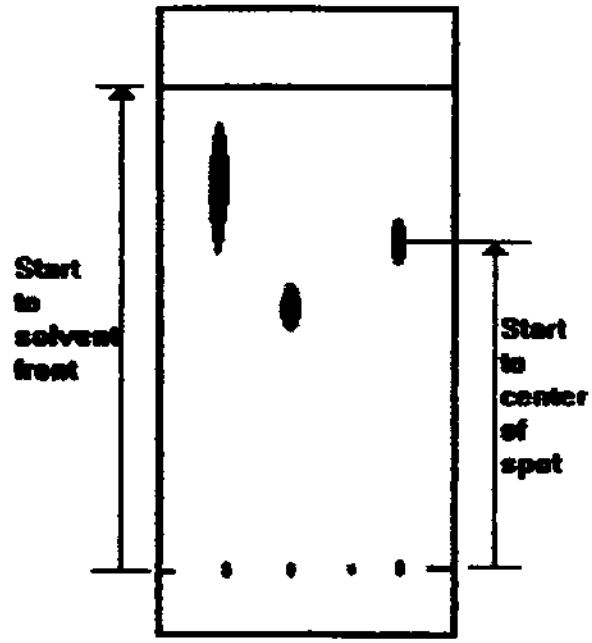
For the location of the separated compounds, the plates were examined by the following methods:

1. Examination under UV light in different wavelength, 254 and 350 cm.
2. The plates were exposed to iodine vapor for several minutes.
3. The plates were sprayed with vanillin-sulphuric acid reagent (1.0%) followed by heating in an oven at 110^0C at for 15 minutes.

Fig 2.5: TLC plate under UV lamp

2.1.4.7 THE R_f VALUE:

Retardation factor (R_f) is the ratio of the distance the compound travels to the solvent front moves.



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

Usually, the R_f value is constant for any given compound and it corresponds to a physical property of the comp.

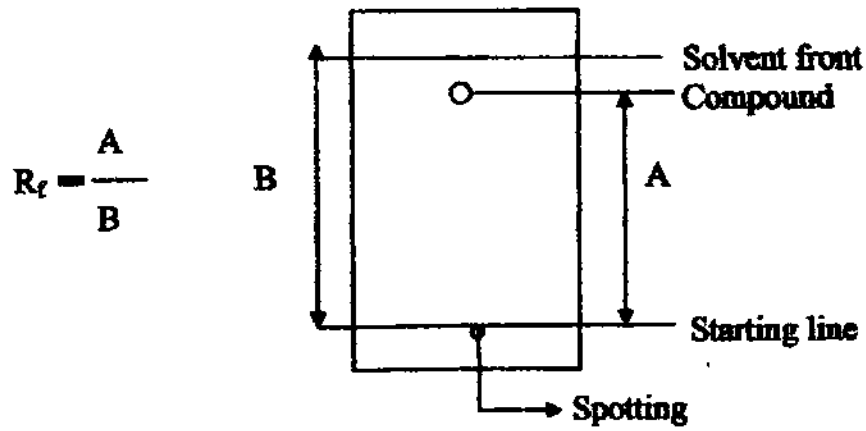


Figure- 2.6: A plate for the calculation of R_f value.

2.1.5 VACUUM LIQUID CHROMATOGRAPHY (VLC)

The concept of vacuum liquid chromatography (VLC) is a relatively recent development in the field of chromatographic separation. It is a column chromatography under reduced pressure and the column is packed with TLC grade silica gel. This method was applied for fractionating the crude extracts in order to obtain either a pure compound or mixture with smaller number of compounds.

A glass column of about 40 cm, length having a system fitted with a water pump and collecting flask at the bottom was taken. Silica gel (G 60, TLC grade) was used as the adsorbent. Silica gel slurry was prepared in petroleum ether or any other suitable solvents. This slurry was packed into hard cake, under an applied vacuum to give a column 3.15 cm in diameter and about 7.0 cm in height. The sample mixture or extract, which to be separated, was dissolved in a minimum volume of suitable solvent. Then small amount of silica-gel (around twice of the amount of sample) was mixed with sample solution. After, through mixing, the solvent of the mixture of sample in silica-gel was evaporated using rotary evaporator. To ensure complete, removal of solvent, the sample- silica gel mixture was kept in a vacuum desiccators. After drying, the mixture was converted into the powder by mortar. The powder was then poured on the top of the packed column to form a layer. Gradient elution with increasing polarity of eluting solvent was used until the more polar component was eluted. These elutes were collected manually in the fraction of different amounts and then the individual fractions were monitored by TLC.

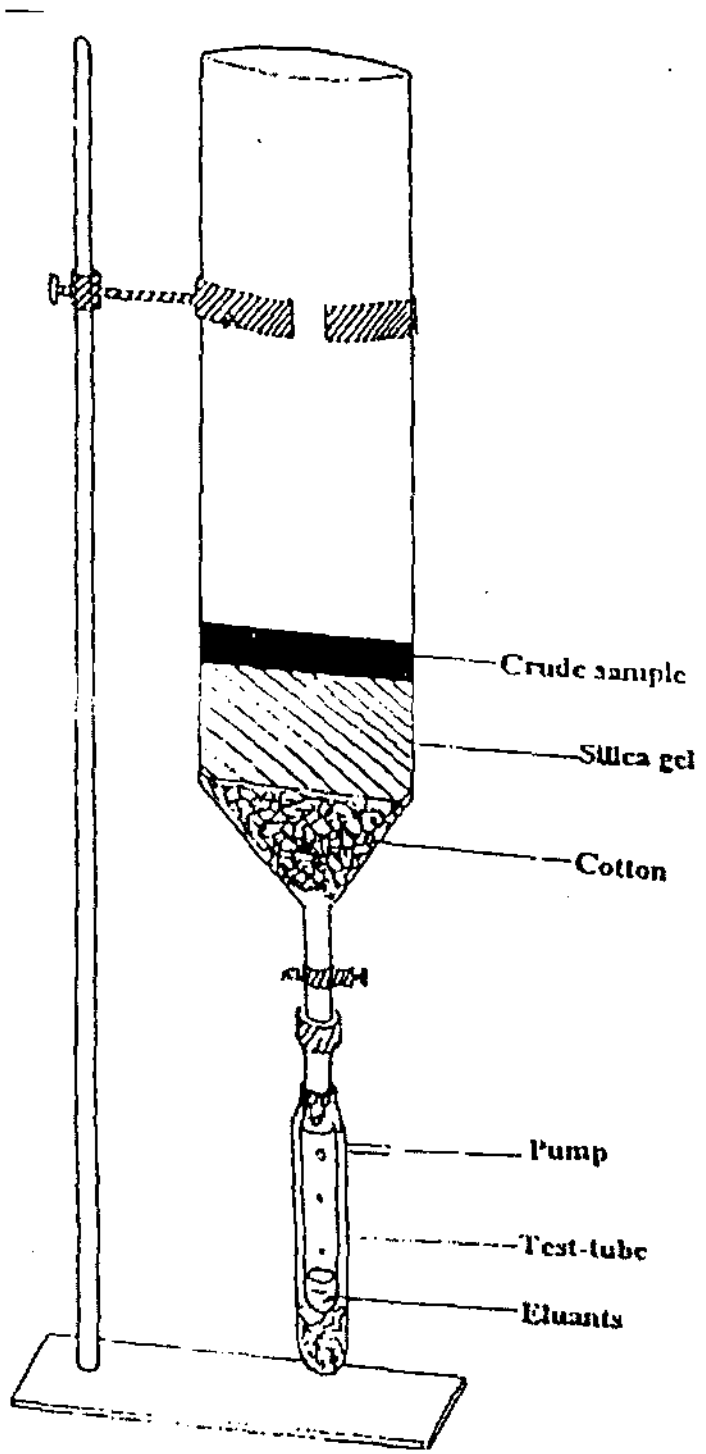


Figure- 2.7: Vacuum liquid chromatography (VLC) apparatus.

2.1.6 CRYSTAILIZTION

Crystallization was employed as a final purification process. A solution of the compound in a minimum volume of the solvent in which it is soluble was prepared in hot condition. It was then left for crystallization. Sometime, a mixture of solvent was used.

2.1.7 SPECTROSCOPIC TECHNIQUES

2.1.7.1 *ULTRA - VIOLET* SPECTROSCOPY *(UV)*

Ultra-violet spectra were recorded using the Shimadzu UV-160A spectrometer. A small amount of the samples of different compounds were dissolved in chloroform or pyridine separately. The solution was taken in quartz cell (1 cm × 1 cm) to recording the spectrum. The main bands (λ_{max}) were recorded as wavelength (nm).

2.1.7.2 INFRA-RED SPECTROSCOPY (IR)

A Shimadzu IR-470 spectrometer was used for recording infrared spectrum. Major bands (ν_{max}) were recorded in wave number (cm^{-1}) as KBr pellets.

2.1.7.3 NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPY

NMR spectra of pure samples extracts were recorded by using ^{1}H-NMR (400 MHz) spectrometer. The spectra were recorded by using $CDCl_3$ and C_5D_5N with tetra methyl silane (TMS) as standard reference.

2.1.7.4 MASS SPECTROSCOPY

Mass spectrum of pure compound was recorded using GC-MS (Agilent Technologies 5975B EI/CI MSD, EU)

2.1.8 DETERMINATION OF MELTING POINT

Melting point (m.p) was determined by using an electrothermal melting point apparatus (Mel-temp), OGAWA SEIKICO, and Japan).

2.1.9 EXTRACTION PROCEDURE USED: All solvent extraction processes were carried out in soxhlet apparatus.

EXPERIMENTAL

2.2 INVESTIGATION OF *Amaranthus spinosus* L.

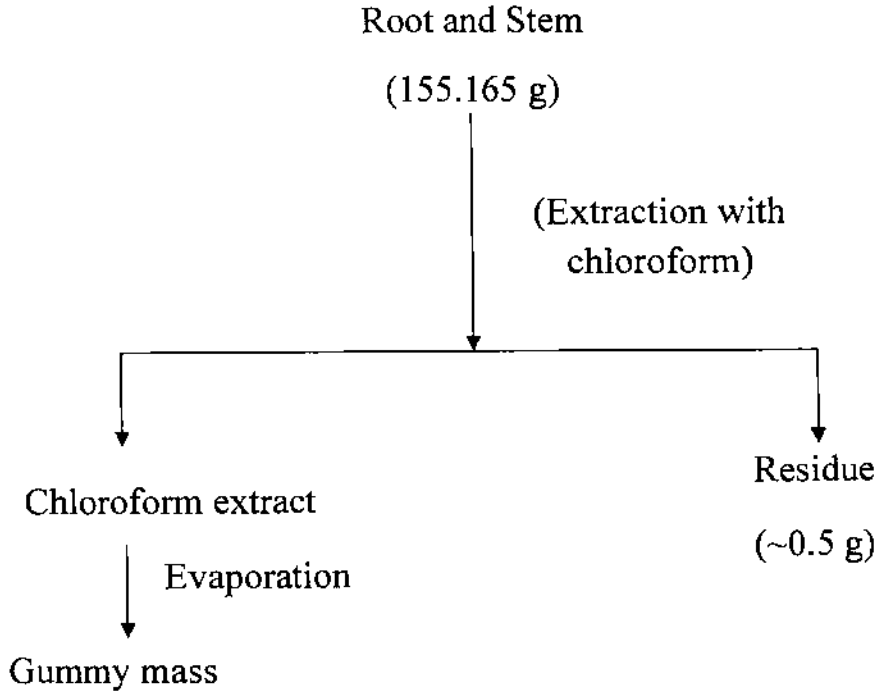
2.2.1 COLLECTION OF THE PLANTS

The plant 'Katanotey' (*Amaranthus spinosus)* was collected from Pabna district. A voucher specimen of this plant was deposited in Botany Department of Dhaka University. The root stem and leaves of this plants were separated and dried under mild sunlight and then at 400^0C in an oven. Afterwards the root & stem were powdered separately (~200 mesh) in a grinding machine. The powder was used throughout the investigation.

2.2.2 EXTACTION AND ISOLATION OF THE COMPOUND FROM THE ROOTS AND STEM OF *Amaranthus spinosus:*

The root and stem (165 g) was extracted with chloroform. The extracts was filtered and concentrated to a small volume separately using a rotary evaporator (Buchi Rotavapor), under reduced pressure. This was shown in the scheme-1.

2.2.3 EXTRACTION SCHEME OF *Amaranthus spinosus* L. (root and stem)



2.2.4 INVESTIGATION OF CHLOROFORM EXTRACTS

2.2.4.1 (a) TLC (Thin Layer Chromatography)

TLC analysis of the chloroform extract showed several spots under UV-light, in iodine chamber and vanillin-sulphuric acid spray on TLC plate.

2.2.4.1 (b) VLC (Vacuum liquid Chromatography)

The gummy mass of Chloroform extract (10.0g) was subjected to VLC and different fractions were collected using different solvent of increasing polarity (Table: 2.1)

Table-2.1:

Number of fractions collected in test tubes from VLC of chloroform extract by using different solvent system.

Solvent system		Amount of solvent (ml)	Number of test tube
Petroleum ether (ml)	Dichloromethane (ml)		
100	0	100	1-21
95	5	100	22-32
90	10	100	33-43
85	15	100	44-53
80	20	100	54-63
75	25	100	64-71
140	60	200	72-85
65	35	100	86-94
60	40	100	95-101
75	75	150	102-113
45	55	100	114-121
40	60	100	122-133
30	70	100	134-140
20	80	100	141-147
10	90	100	148-154
5	45	50	155-158

Table-2.2: Number of fractions collected in test tubes of chloroform extracts by using different solvents.

Solvent system			Amount of Solvents (ml)	Number of test tube
Dichloromethane (ml)	Ethyl acetate (ml)	Methanol (ml)		
100	0	0	100	159-165
90	10	0	100	166-172
80	20	0	100	173-180
70	30	0	100	181-187
60	40	0	100	188-194
0	100	0	100	195-200
0	0	130	130	201-210

2.2.4.2 SCREENING OF THE FRACTIONS:

Each of the fractions was monitored by TLC and the fractions of similar behaviors were combined together and marked as T_1, T_2, T_3, T_4, T_5, T_6, T_7, T_8, T_9, T_{10}, T_{11}, T_{12}, T_{13}, T_{14}, T_{15}, T_{16}, T_{17}, T_{18}, T_{19}, T_{20}, T_{21}, T_{22} and this is given in Table-2.3.

Table-2.3: Screening of the fractions by similar TLC pattern

Test-tube numbers	Fractions	TLC pattern
1-21	T_1	No spot was found
22-32	T_2	No spot was found
33-43	T_3	No spot was found
44-53	T_4	Two spot with tailing
54-63	T_5	Two spot with tailing
65-71	T_6	Three spot with tailing
72-85	T_7	Two spot with tailing
86-94	T_8	One spot with tailing
95-102	T_9	Two spot with tailing
103-114	T_{10}	One spot with tailing
115-120	T_{11}	Single spot with no tailing
121-128	T_{12}	Two spot
129-139	T_{13}	Single spot with no tailing
140-147	T_{14}	Single spot with no tailing
147-158	T_{15}	One spot with tailing
159-165	T_{16}	One spot with tailing
166-172	T_{17}	Two spot with tailing
173-180	T_{18}	Two spot with tailing
181-187	T_{19}	Two spot with tailing
188-194	T_{20}	One spot with tailing
195-200	T_{21}	No spot was found
201-210	T_{22}	No spot was found

2.2.4.3 ANALYSIS OF THE FRACTIONS:

TLC analysis of the fractions showed T_1, T_2, T_3, T_{21}, T_{22},contains no observable compounds T_4, T_5, T_6, T_7,T_8, T_9, T_{10}, T_{12}, and T_{11}, T_{15}, T_{16}, T_{17}, T_{18}, T_{19}, T_{20} were mixture of the compound and T_{11}, T_{13},and T_{14} were found to be single compounds. So T_{11}, T_{13}, and T_{14} were analyzed.

2.2.4.3.1 Analysis of fraction T_{11}:

Fraction T_{11}, (~ 50 ml) was left undisturbed at room temperature for several days. A white crystalline solid was obtained in both. The crystals were separated from mother liquor and was washed with different solvents. Its TLC study also showed a single spot in different solvent systems. It was designated as A_1,

2.2.4.3.1.1 PROPERTIES OF THE ISOLATED COMPOUNDS (A_1)

2.2.4.3.1.2 Properties of A_1,

Physical state	:	White crystalline solid.
Solubility	:	Moderately soluble in petroleum-ether and highly soluble in chloroform.
R_f value	:	0.40 (in 60% dichloromethane and 40% petroleum-ether)
Melting point	:	130-132^{0}C
Amount	:	~ 6 mg.

Spectral characteristics:

IR ν_{max} cm^{-1} in (KBr pellet) (from fig-3.1) : 3450, 2900, 1660, 1480 cm^{-1}.

3450 cm^{-1}	:	Stretching for -OH
2900 cm^{-1}	:	Stretching for $-CH_3$
1660 cm^{-1}	:	Stretching for C=C
1480 cm^{-1}	:	Bending for $-CH_2-$

^{1}H-NMR spectrum (400 MHz, $CDCl_3$) (From figure-3.2, 3.3, 3.4) : The compound A_1 had important chemical shift (δ value) at 0.546, 0.853, 0.873, 0.961, 1.802, (1.013-2.27), 3.58, 5.34 ppm.

^{13}C-NMR spectrum (400MHz, $CDCl_3$) (From figure-3.5 - 3.10) : The compound A_1 had important chemical shift ((δ value) at 37.20, 31.54, 71.10, 40.33, 138.17, 117.50, 31.90, 31.54, 51.29, 38.06, 21.60, 39.52, 43.33, 55.60, 55.18, 28.33, 55.18, 12.08, 21.60, 38.06, 19.03, 31.90, 25.41, 49.52, 29.37, 21.39, 21.08, 23.05, 12.24 ppm.

2.2.4.3.2 Analysis of T_{13}: The fraction T_{13} was allowed to stand for several days, when a write crystalline compound appeared and it was marked as A_2.

2.2.4.3.2.1 Properties of A_2:

Physical state : White crystalline solid.

Solubility : Moderately soluble in petroleum-ether and highly soluble in chloroform.

R_f value : 0.60 (in 60% dichloromethane and 40% petroleum-ether)

Melting point : 203-205^{0}C

Spectral characteristics:

IR ν_{max} cm^{-1} in (KBr pellet) (From figure-3.11) : 3400, 2900, 1640, 1460 cm^{-1}

3400 cm^{-1}	:	Stretching for -OH
2900 cm^{-1}	:	Stretching for $-CH_3$
1640 cm^{-1}	:	Stretching for C=C
1460 cm^{-1}	:	Bending for $-CH_2-$

2.2.4.3.3 Analysis of T_{14}: The fraction T_{14} yielded a white crystalline solid on standing for 48 hours it was marked as A_3.

2.2.4.3.3.1 Properties of A_3 :

Physical state : White crystalline solid.

Solubility : Moderately soluble in petroleum-ether and highly soluble in chloroform.

R_f value : 0.50 (in 50% dichloromethane and 50% petroleum-ether)

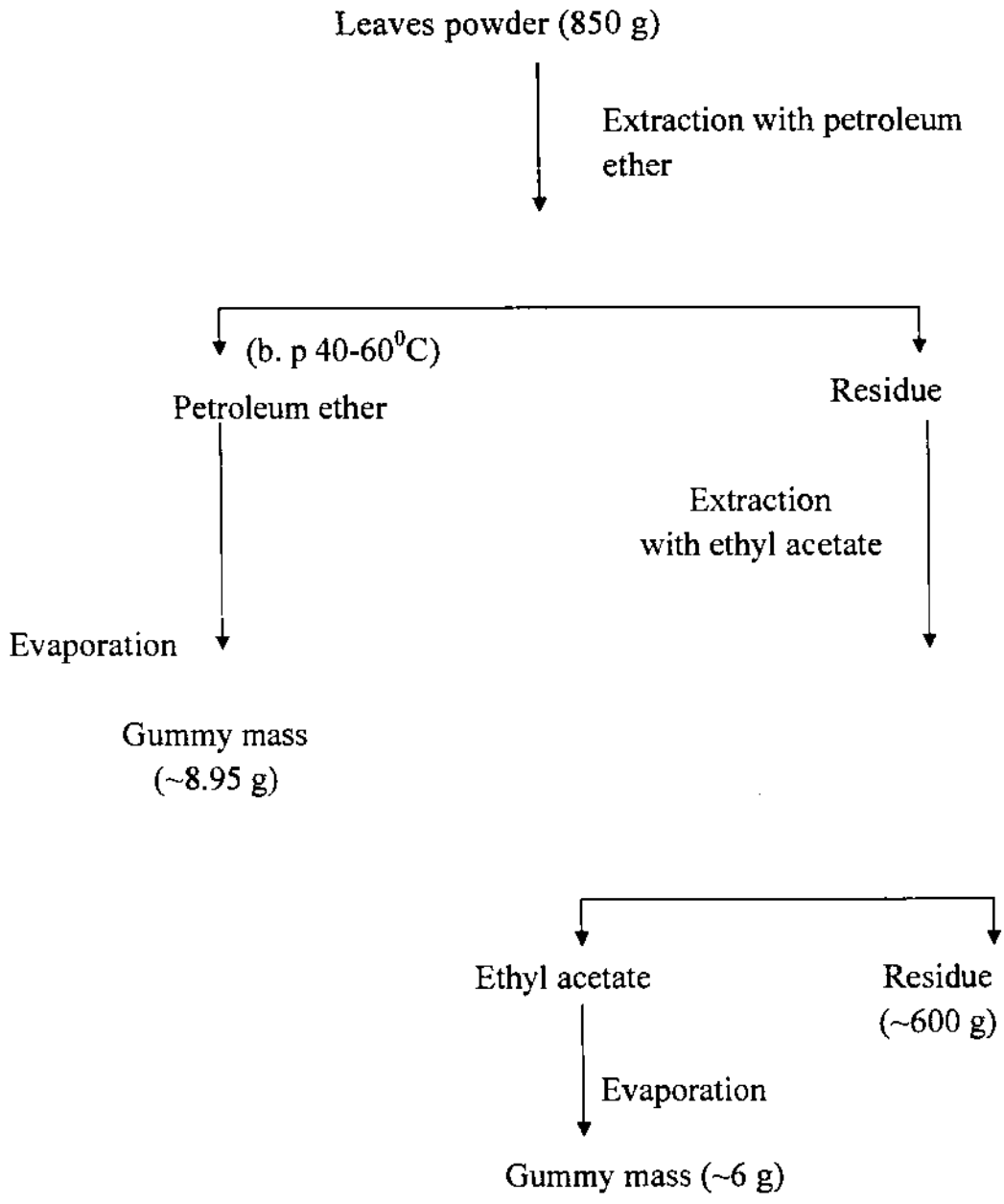
Melting point: 175-180^{0}C

Spectral characteristics:

IR νmax cm^{-1} in (KBr pellet)(From figure-3.12) : 3400, 1460, 1360 cm^{-1}

3400 cm^{-1}	:	Stretching for cm^{-1}
2900 cm^{-1}	:	Stretching for -OH
1460 cm^{-1}	:	Bending for $-CH_2-$
1360 cm^{-1}	:	Stretching for -C-O

2.3 EXTRACTION SCHEME OF *Anaranthus spinosus (leaves)*



Scheme -2: Extraction procedure of *Amaranthus spinosus (leaves)*

INVESTIGATION OF PETROLEUM-ETHER (b.p 40-60^{0}C) EXTRACT

2.3.1 TLC (Thin Layer Chromatography)

TLC analysis of the petroleum-ether (b. p 40-60^{0}C) extract showed several spots under UV-light, in iodine chamber and vanillin-sulfuric acid spray on TLC plate.

2.3.2 VLC (Vacuum Liquid Chromatography)

The petroleum-ether extract was concentrated by 'rotary evaporator'. The concentrated pet-ether extract (~20g) was mixed with TLC grade silica-gel ($60GF_{254}$). This sample was placed on the top of the bed of silica-gel packed column (VLC). The column was then eluted with petroleum ether followed by mixtures of petroleum ether and ethyl-acetate of increasing polarity. Solvent systems used as mobile phases in the analysis of petroleum ether extract were listed in Table-2.4:

Table -2.4:

Number of fractions collected in test tubes from VLC of petroleum ether extract by using different solvent system.

Solvent systems		Amount of solvents (ml)	Number of test-tubes
Petroleum ether %	Ethyl acetate %		
100	0	200	1-26
99	1	200	27-34
98	2	200	35-43
95	5	200	44-54
90	10	200	55-63
85	15	200	64-71
80	20	200	72-79
75	25	200	80-88
70	30	200	89-109
60	40	150	110-118
50	50	200	119-131

2.3.3 SCREENING OF THE FRACTIONS:

Each of the fractions were monitored by TLC and the fractions of similar behaviors were combined together and marked as C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14} and C_{15} and this is given in Table-2.5

Table-2.5

Screening of the fractions by the similar TLC pattern.

Test-tube Numbers	Fractions	TLC pattern
1-7	C_1	One spot with no tailing.
8-15	C_2	Two spots with tailing.
16-19	C_3	Two spots with tailing.
20-27	C_4	Two spots with tailing.
28-37	C_5	Two spots
38-43	C_6	Two spots with tailing.
44-52	C_7	Two spots with tailing.
53-65	C_8	Three spots with tailing.
66-82	C_9	One spots with tailing.
83-97	C_{10}	Two spots with tailing.
98-109	C_{11}	Two spots with tailing.
110-116	C_{12}	Two spots with tailing.
117-120	C_{13}	One spots with tailing.
121-125	C_{14}	Two spots with tailing.
126-131	C_{15}	One spots with tailing of impurities.

2.3.4 ANALYSIS OF THE FRACTIONS:

TLC analysis of the Fraction showed C_1 and C_9 as single compound and C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_{10}, C_{11}, C_{12}, C_{13} and C_{14} were mixture of compounds but C_{15} was found to be a single compound with trace impurity. So C_1 and C_9 were analyzed.

2.3.4.1 Analysis of fraction C_1:

Fraction C_1 (~40 ml) was left undisturbed at room temperature for several days. A white crystalline powder was obtained. The crystals were separated from mother liquor and purified by washing with different solvents. The powder was washed with petroleum ether very well. Its TLC study also showed a single spot in different solvent systems. It was designated as V_1.

2.3.4.2 Analysis of fraction C_9:

The fraction C_9, (~45ml) was concentrated in a rotary evaporator and was left undisturbed at room temperature. After several days a off-white crystal substance was obtained. The crystals were washed with petroleum ether very well. A needle shaped white crystals were settled down which were separated from mother liquor. It's TLC study also showed a single spot (R_f value = 0.68) in solvent system (Pet-ether: ethyl acetate=3:1). The spot turned into violet color by spraying vanillin-sulfuric acid. It was also iodine active but not UV active. It was designated as V_2.

2.3.5 PROPERTIES OF THE ISOLATED COMPOUNDS

2.3.5.1 Properties of V_1:

Physical state : White crystalline powder.

Solubility : Moderately soluble in pet-ether, Methanol and highly. Soluble in ethyl-acetate and chloroform.

R_f value : 0.50 (over silica gel, 60 GF_{254}, pet-ether: Ethyl acetate=3:1 as the mobile phase).

Melting Point : 152-154^{0}c

Amount : ~19mg.

Spectral characteristics:

IRγ_{max} KBrcm^1 (Fig-3.13): 3420, 3050, 2930, 2850, 1640, 1450, 1370, 1040, 970, 840, 820

^{1}H-NMR spectrum (Fig-3.14 -3.15):

δ 0.540 (s,3H in C-18)

δ 0.795 (broad s, 3H in C-27)

δ underneath at 0.795 (3H in C-29)

δ 0.814 (s, 3H in C-19)

δ 0.847 (d, J=5.9 Hz in C-26)

δ 1.024(d, J=6.67 Hz in C-21) 1.40-2.0 (m, for methine & methylene protons

δ 2.009 (s, 1H in-OH)

δ 3.584 (m, 1H at C-3) δ 5.024 (m, 1H, H-7)

δ 5.045-5.119 (m, 2H, H-22 & H-23)

^{13}C-NMR spectrum (400 MHz, $CDCl_3$) (Fig - 3.16 - 3.19):

37.18, 139.59, 23.11, 138.18, 31.52, 49.49, 28.52, 129.48, 71.09, 34.30, 55.94, 51.28, 39.03, 21.60, 12.15, 32.01, 40.30, 39.51, 3.11,

21.41, 29.67, 43.32, 40.84, 19.02, 17.49, 55.21, 21.39, 25.41, 12.31.

2.3.5.2 Properties of V_2:

Physical state : Off-white needle shaped crystals;

Solubility : freely soluble in chloroform;

R_f value : 0.55 (over silica gel, 60 GF254, Petroleum-ether: Ethyl-acetate=3:1, as a mobile phase).

Melting Point : 61-62^{0}C;

Amount : ~ 26 mg

Spectral Characteristics:

IRγ_{max} KBr cm^{-1}(Fig-3.20): 3450, 2950, 2850, 1430, 1350, 1050, 930;

^{1}H-NMR Spectrum (400 MHz, $CDCl_3$) (Fig-3.21, 3.22): 0.871 (3H; t; J=6.68 Hz; Me), 1.245 (34H, s; for-CH2-protons), 1.603 (2H; t; for-CH2-protons), 4.061 (1H, s, -OH)

^{13}C-NMR Spectrum (400MHz, $CDCl_3$) (Fig-3.23 - 3.25): 14.137, 22.719, 25.072, 25.973, 28.692, 29.198, 29.296, 29.388, 29.508, 29.728 (seven carbon together), 31.956, 34.462, 64.482,

EIMS: M^+ m/z (Rel. int) (Fig-3.26, 3.27): [M+1] 285.30 (1.67), 257.35 (15.00), 256.00 (2.96), 167.10 (1.13), 153.10 (2.08), 139.10 (2.58), 129.10 (4.28), 112.05 (2.60), 111.20 (11.96), 97.15 (24.52), 83.10 (29.12), 71.15 (38.00), 69.10 (30.11), 57.10 (100.00).

2.4. INVESTIGATION OF ETHYL- ACETATE EXTRACT OF LEAVES

2.4.1 THIN LAYER CHROMATOGRAPHY (TLC)

The ethyl acetate extract of the leaves powder was analyzed by thin layer chromatography (TLC). Analysis of this extract showed several dull reddish spots under UV-light and the same spot was found to be greenish in broad daylight.

2.4.2 VACUUM LIQUID CHROMATOGRAPHY (VLC)

The ethyl acetate extract was concentrated to a dry mass (8.5 gm) in a rotary vacuum evaporator (Buchi). The dry mass of ethyl-acetate extract (5gm) was dissolved in a small volume of ethyl acetate and mixed with TLC grade silica-gel (60 GF_{254}) Powder.

The mixture was evaporated to dry powder end the sample was placed on the top of the bed of silica-gel packed column (VLC). The column was eluted first with 100% petroleum ether (b.p. 40^0-60^0C), followed by mixtures of petroleum ether, dichloromethane and increasing amount of ethyl acetate and finally with 50% methanol. The elutes were collected in test tube with an amount of about 15 ml in each.

The different ratios of the solvent used as an elutent are given in Table: 2.6

Table-2.6: The solvent system used for separation of the concentrated ethyl acetate extract by vacuum liquid chromatography (VLC):

Solvent system				Amount of solvent (ml)	Number of test tube
Petroleum ether (%)	Dichloro-methane (DMM) (%)	Ethyl acetate (%)	Methanol (%)		
100%	0	0	0	350	1-16
95%	5	0	0	200	17-25
90	10	0	0	250	26-36
85	15	0	0	200	37-45
80	20	0	0	200	46-53
70	30	0	0	200	46-53
60	40	0	0	200	63-72
55	45	0	0	200	73-80
45	55	0	0	200	81-91
30	70	0	0	200	92-100
0	100	0	0	300	101-115
0	95	5	0	200	116-121
0	90	10	0	200	122-130
0	80	20	0	200	131-142
0	70	30	0	100	143-148

Solvent system				Amount of solvent (ml)	Number of test tube
Petroleum ether (%)	Dichloro-methane (DMM) (%)	Ethyl acetate (%)	Methanol (%)		
0	60	40	0	100	149-154
0	50	50	0	200	155-164
0	40	60	0	200	165-173
0	30	70	0	200	174-183
0	0	100	0	200	184-192
0	0	90	10	200	193-200
0	0	80	20	100	201-205
0	0	70	30	100	206-211
0	0	50	50	100	212-220

2.4.3 SCREENING OF THE FRACTION:

Each of the fractions were monitored by TLC and the fractions of similar behaviors was combined together and marked as E_1, E_2, E_3, E_4, E_5, E_6, E_7, E_8, E_9 and E_{10}.

All the fractions from E_1 to E_{10} were concentrated to a small volume (5 ml) and small amount of CH_2Cl_2 was added to it for crystallization. All the fractions were allowed to stand undisturbed for several days at room temperature but no crystal was found.

On the basis of the similarity in TLC pattern, the fraction E_1 and E_2 were again combined together and was marked as F_1. Similarly, fractions E_3 to E_4 were combined together and marked as F_2. E_5 and E_6 were marked as F_3 and F_4 respectively. Fractions E_7 to E_{10} and the rest of the conical flasks were mixed together and marked as F_5 and this is given in the table- 2.7.

Table-2.7: Screening of the fractions by the similar TLC pattern.

Conical flasks	Fractions	TLC pattern
E_1-E_2	F_1	Four spots with tailing
E_3-E_4	F_2	Two spots with tailing
E_5	F_3	Five spots with chlorophyll.
E_6	F_4	Three spots with tailing
E_7-E_{10}	F_5	Mixture of compounds with chlorophyll.

2.4.4 ANALYSIS OF THE FRACTIONS:

Fraction F_1, F_3 and F_5 were analyzed but no satisfactory results were obtained. F_2 was analyzed and fond to have, two spots in TLC plate. These two spots were separated by PTLC and these were allowed to stand when two crystalline compounds were obtained from two spots. They were marked as H_1 and H_2.

2.4.3.1 PROPERTIES OF H_1.

Physical state	:	White solid.
Solubility	:	Soluble in dichloromethane, chloroform and ethyl acetate.
R_f value	:	0.68 (over silica gel 60 GF_{254}, petroleum ether: ethyl acetate=4:1).
Melting point	:	32-35^{0}C.
Amount	:	~1 mg.

2.4.3.2 PROPERTIES OF H_2

Physical state	:	Off-white crystalline solid.
Solubility	:	Soluble in dichloromethane, chloroform, ethyl acetate and methanol.
R_f value	:	0.50 (Over silica gel 60 GF 254 EA: MeOH=9:1).
Melting point	:	62-64^{0}C.
Amount	:	~3 mg.

Spectral Characteristics:

IR ν_{max} KBr (Fig-3.28): The compound H_2 had important frequencies of absorption at 3420, 2930, 2850, 1690, 1640, 1450 and 1370 cm^{-1}.

^{1}H-NMR (400 MHz, in $CDCL_3$, using TMS as standard) (Fig 3.29-3.30):

δ 0.86-0.87 (t, 3H at C22).

δ 1.25 (m. 36 H and C-4-c-21)

δ 1.624 (m, at, at C-3)

δ 2.32 - 2.35 (t, 2Hand C-2)

2.4.3.3 ANALYSIS OF THE FRACTION F_4

Fraction F_4 was analyzed and three spots were found with tailing, indicating the presence three compounds in that fraction. In order to separate those three compounds, preparative thin layer chromatography technique was performed when three separated fractions were obtained. All the three fractions were kept undisturbed for several days when each of them yielded crystalline compound and they all named as M_1, M_2 and M_3 .

2.4.3.4 CHARACTERISTICS OF M_1

Physical state	:	Yellow gammy substance.
Solubility	:	Soluble in dichloromethane, chloroform and ethyl acetate.
R_f value	:	0.55 (over silica gel, 60 GF_{254}, petroleum ether: ethyl acetate=4:1).
Melting point	:	110-112^{0}C.
Amount	:	~1 mg.

2.4.3.5 CHARACTERISTICS OF COMPOUND M_2

Physical state	:	Reddish semi-solid.
Solubility	:	Soluble in dichloromethane, chloroform and ethyl acetate.
R_f value	:	0.50 (Over silica gel, 60 GF_{254}, petroleum ether: ethyl acetate=4:1).
Melting point	:	80-82^{0}C.
Amount	:	~1 mg.

SPECTRAL CHARACTERISTICS OF M_2:

IR ν_{max} KBr (Fig-3.39): The compound M_2 had important frequencies of absorption at 3420, 2930, 2850, 1725, 1650, 1450, 1375, 1225, 1020 and 810 cm^{-1}.

^{1}H-NMR: was not done due to insufficient sample.

2.4.3.6 STUDY ON COMPOUND M_3

Physical state : Yellow semi-solid.

Solubility : Soluble in dichloromethane, chloroform and ethyl acetate and methanol.

R_f value : 0:62 (Over silica gel, 60 GF_{254}, petroleum ether: dichloromethane = 4:1).

Amount : ~4 mg.

SPECTRAL CHARACTERISTICS:

IR ν_{max} KBr (Fig-3.31): The compound M_3 had important frequencies of absorption at 3450, 3050, 2930, 2850, 1725-1700, 1640, 1460, 1375, 1250-1050 and 810 cm^{-1}.

^{1}H-NMR (400 MHz, $CDCl_3$, TMS) (Fig-3.32 - 3.34)

δ 1.99 ppm (t, 1H at C-1)

δ .82 ppm (t, 3H at C-9)

δ 1.58 ppm and δ 2.27 ppm (t, two non-equivalent proton at C-6)

δ 1.67 ppm (Broad 's' at CH_2 Proton at C-5).

δ 2.01 ppm ('s' 3H at C-10).

δ 3.63 ppm ('s' hydroxyl proton).

δ 4.1 ppm ('q' 2H at C-8).

δ 4.5 ppm ('d' an oxy methine proton at C-2).

^{13}C-NMR spectrum (400 MHz, $CDCl_3$): (Fig-3.35-3.37)

δC 173.96, 142.78, 118.0, 96.2, 61.46, 32.80, 39.87, 37.44, 21.06, 19.75 ppm

CHAPTER THREE

RESULT & DISCUSSION

3. RESULTS AND DISCUSSION

3.1 Sample preparation:

The root and stem of the plants were separated and dried under mild sunlight and then at 400^0C in an oven. Afterwards the root & stem were powdered separately (~200 mesh) in a grinding machine. The powder was used throughout the investigation.

3.2 Solvent extraction: The root and stem powder was extracted with chloroform. The extract was filtered and concentrated to a small volume separately using a Buchii Rotavapor under reduced pressure.

3.3 Isolation and purification of compounds by chromatographic methods:

By using column chromatography the compound was isolated using different eluting solvents to obtain. It was collected in different test tubes and purified. From this analysis three fractions T_{11}, T_{13} and T_{14} were appeared to be pure.

3.4 SPECTROSCOPIC ANALYSIS OF COMPOUND A_1:

3.4.1 IR spectroscopic analysis [34, 35]: IR ν_{max} KBr cm^{-1}

The IR spectrum of the compound A_1 showed an absorption band at 3450 cm^{-1} indicating the presence of -OH group. The band at 2900 cm^{-1} and 2850 cm^{-1} were due to the presence of aliphatic C-H stretching of either both $-CH_3$, $-CH_2$ or >CH group. The peak at 1640 cm^{-1} was suggestive the presence of >C=C< group. The two peaks at 1416 cm^{-1} and 1370 cm^{-1} were due to the presence of $-CH_2-$ and $-CH_3$ groups, respectively. The absorption band at 1100 cm^{-1} and 1040 cm^{-1} were suggestive of C-O bonding. The peaks at 830 cm^{-1} and 800 cm^{-1} were indicative of the presence of >C=C-H.

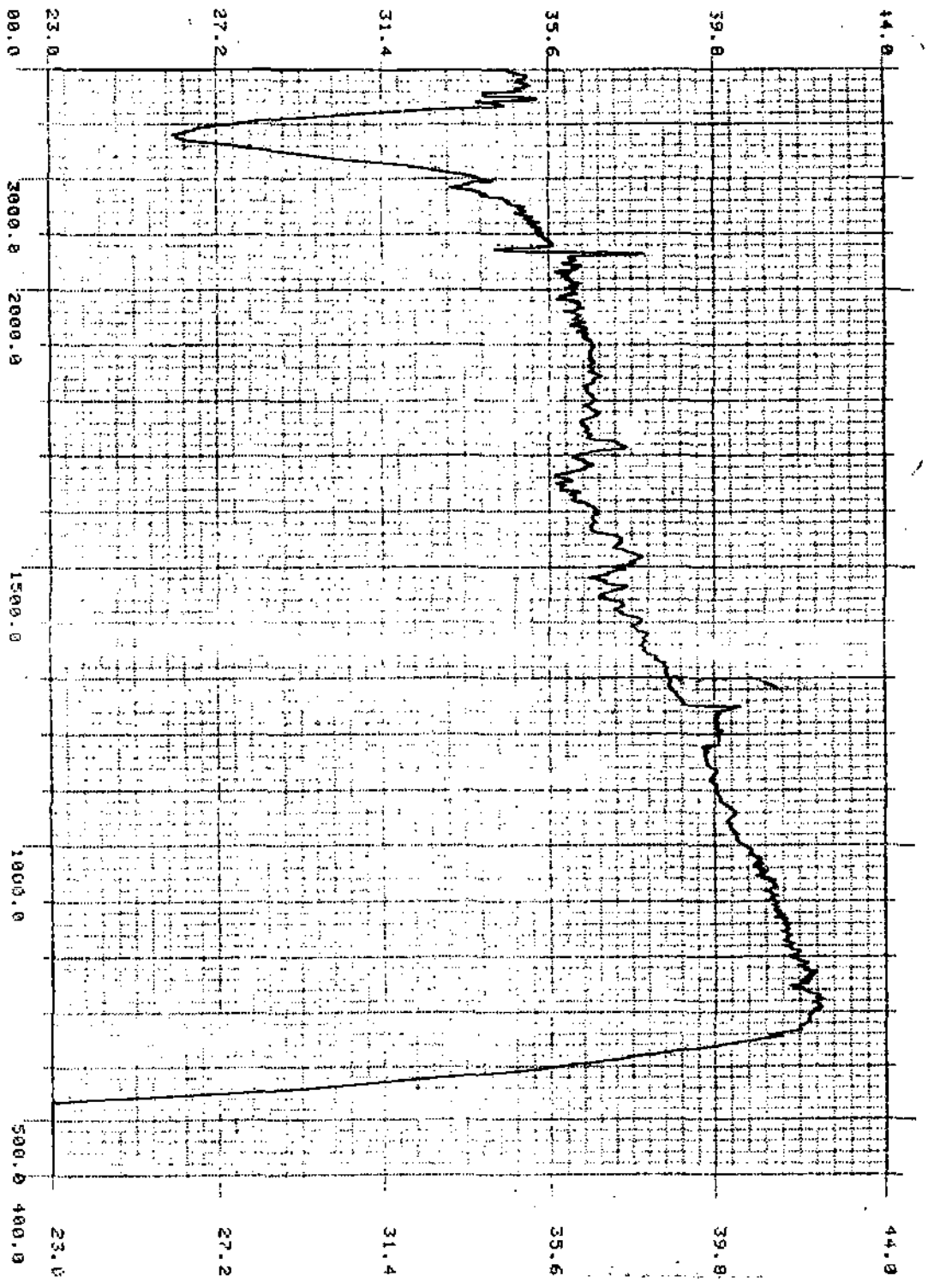
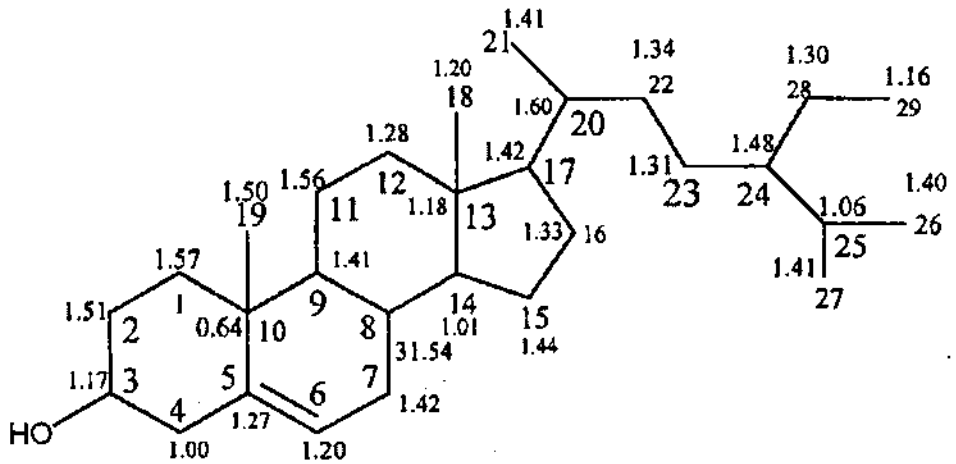


Fig-3.1: IR of Compound A_1

3.4.2 ^{1}H-NMR Spectroscopic analysis:

The ^{1}H-NMR (400 MHz, in $CDCl_3$) spectrum of isolated compound A_1 had two sharp singlet at 0.546 and 0.961 ppm typical for the presence of methyl protons (18-H_3 & 19-H_3) at C-13 and C-10 respectively[36]. The spectrum had a multiplate at 3.58 ppm indicative of the presence of oxymethine proton (3α-H). The distorted multiplet at 5.34 ppm was indicative of the presence of olefinic proton (6-H) at C-6. The three doublets at δ 0.873 ppm (d,J = 6.4 Hz, 21-H_3), at δ 0.837 ppm (d, J=7.2 Hz, 26-H_3) and at δ 0.801 ppm (d, J=8.8 Hz, 27-H_3) were due to the presence of methyl protons at C-20 and C-25 respectively. The spectrum had a triplet at 0.853 ppm (t, 29-Hz) was due to the presence of methyl protons at C-28. The spectrum had a broad singlet at 1.802ppm which was due to the presence of hydroxyl (3β-OH) proton at C-3. The other signals of the spectrum (m, d, t, s) between 1.013-2.27 ppm were due to the presence of different methylene (-CH_2-) and methane (>CH-) protons[36].



β-Sitosterol

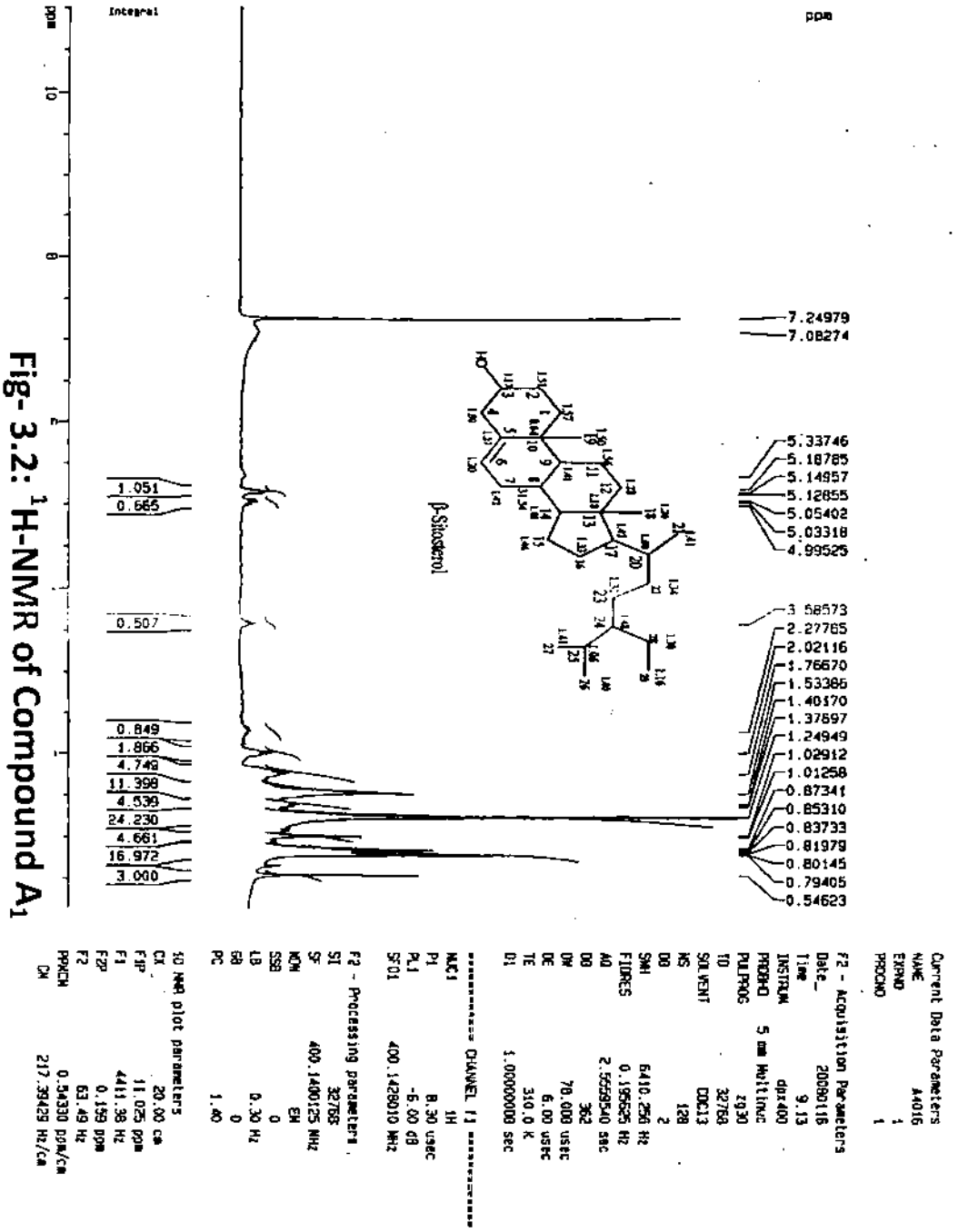


Fig- 3.2: ^{1}H-NMR of Compound A_1

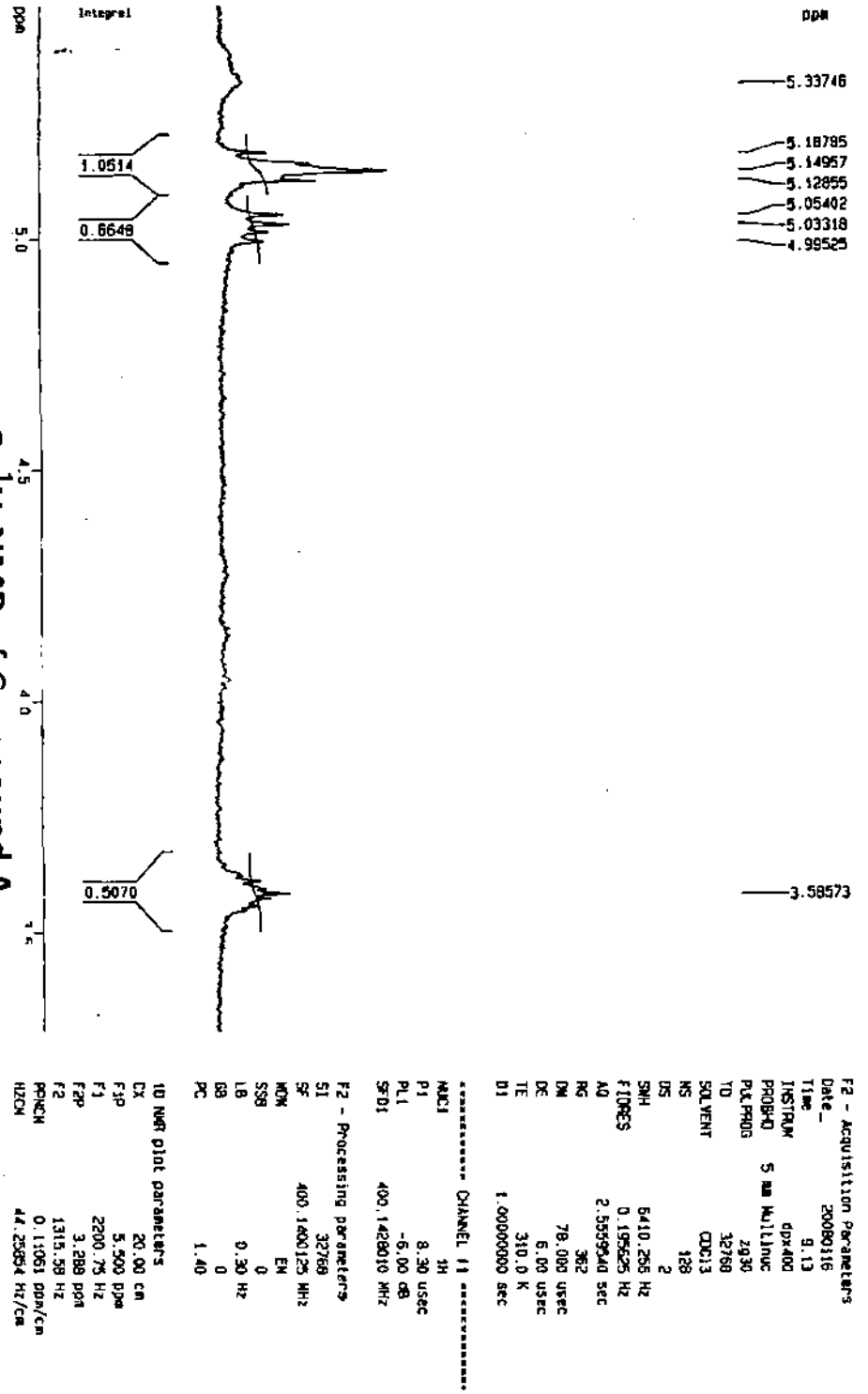


Fig- 3.3: ^{1}H-NMR of Compound A_1

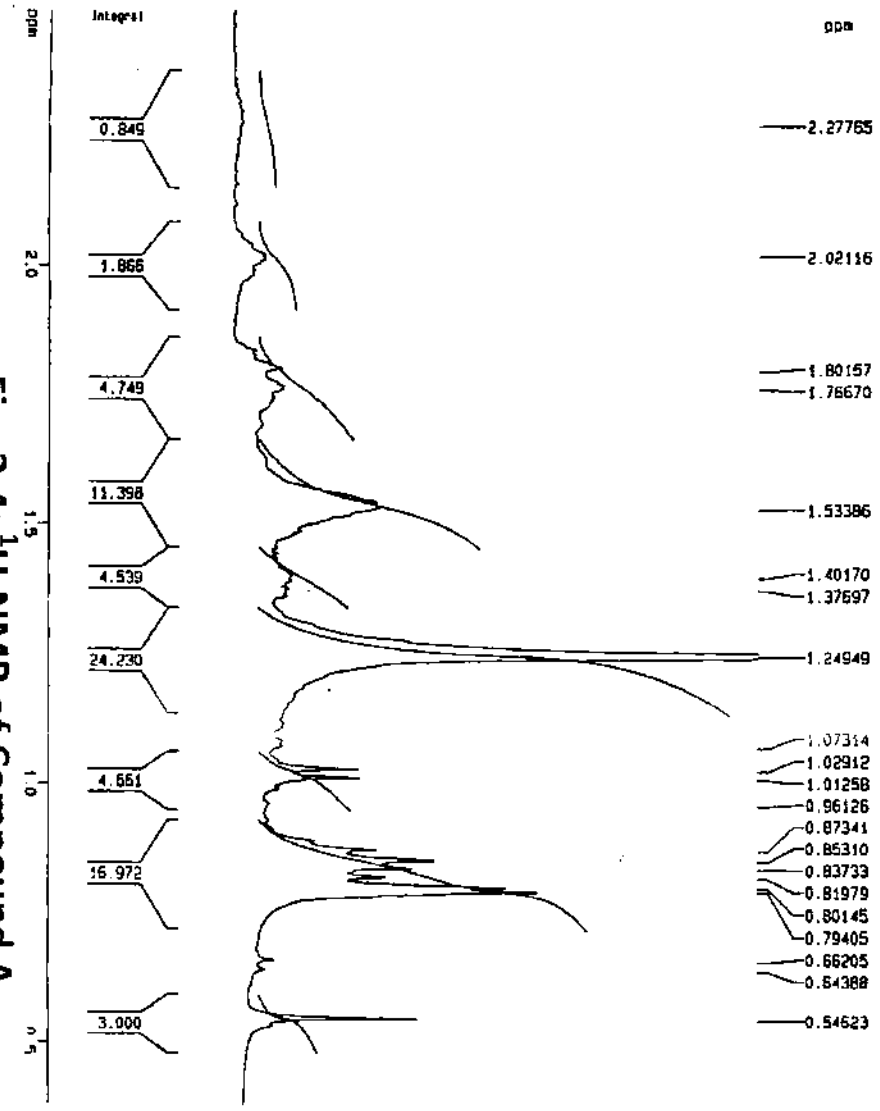


Fig- 3.4: ^{1}H-NMR of Compound A_1

Table 3.1: Comparative ^1H - NMR spectral data of isolated compound A_1 with the published[36] data of β- sitosterol.

Protons	δ value of the isolated compound (ppm)	Published[36] δ value of Acetate of β-sitosterol (ppm)
18-H3	0.546, s	0.676, s
19-H3	0.961, s	1.017, s
21-H3	0.873 (d, J=6, 4Hz)	0.918 (d, J= 6.5Hz)
26-H3	0.837 (d, J=7.2Hz)	0.831 (d, J=6.8Hz)
27-H3	0.801 (d, J=8.8Hz)	0.809 (d, J=7.4Hz)
29-H3	0.853, t	0.841 (d, J=7.2Hz)
3α-H3	3.59, m	4.6, m
6-H	5.34 distorted peak	5.38, m
3β-OH (1H)	1.802, broad singlet	2.021 (s)
Methylene & Methane protons	1.013-2.27 (m, d, t, s)	- -

3.4.3 ^{13}C-NMR Spectroscopy:

The ^{13}C-NMR spectrum[37] of isolated compound A_1 showed the presence of twenty nine (29) carbon signals. Among them, 2- signals were assignable to sp^2 carbons, 11 signals to methylene carbons, 8 signals to methine carbons, 6 signals to methyl carbons and 2 signals for quaternary carbons. The signals at δ 138.166 & 117.499 ppm were due to the olefinic carbons and signals at δ 37.20 and δ 43.33 ppm were assignable to quaternary carbons. The signals at δ 12.08, 19.03, 21.03, 21.08, 21.39 and 12.24 ppm were due to the presence of six methyl carbons and the signals at δ 37.20, 31.90, 42.33, 32.90, 21.60, 39.52, 25.41, 28.33, 31.54, 25.41 & 23.22 ppm were due to the presence of eleven methylene carbons. The signals at δ 71.11, 31.90, 49.52, 55.18, 55.98, 38.39, 43.33 & 29.37 ppm were due to the presence of eight methine carbons. The signal at δ 71.11 was due to the oxymethine carbon.

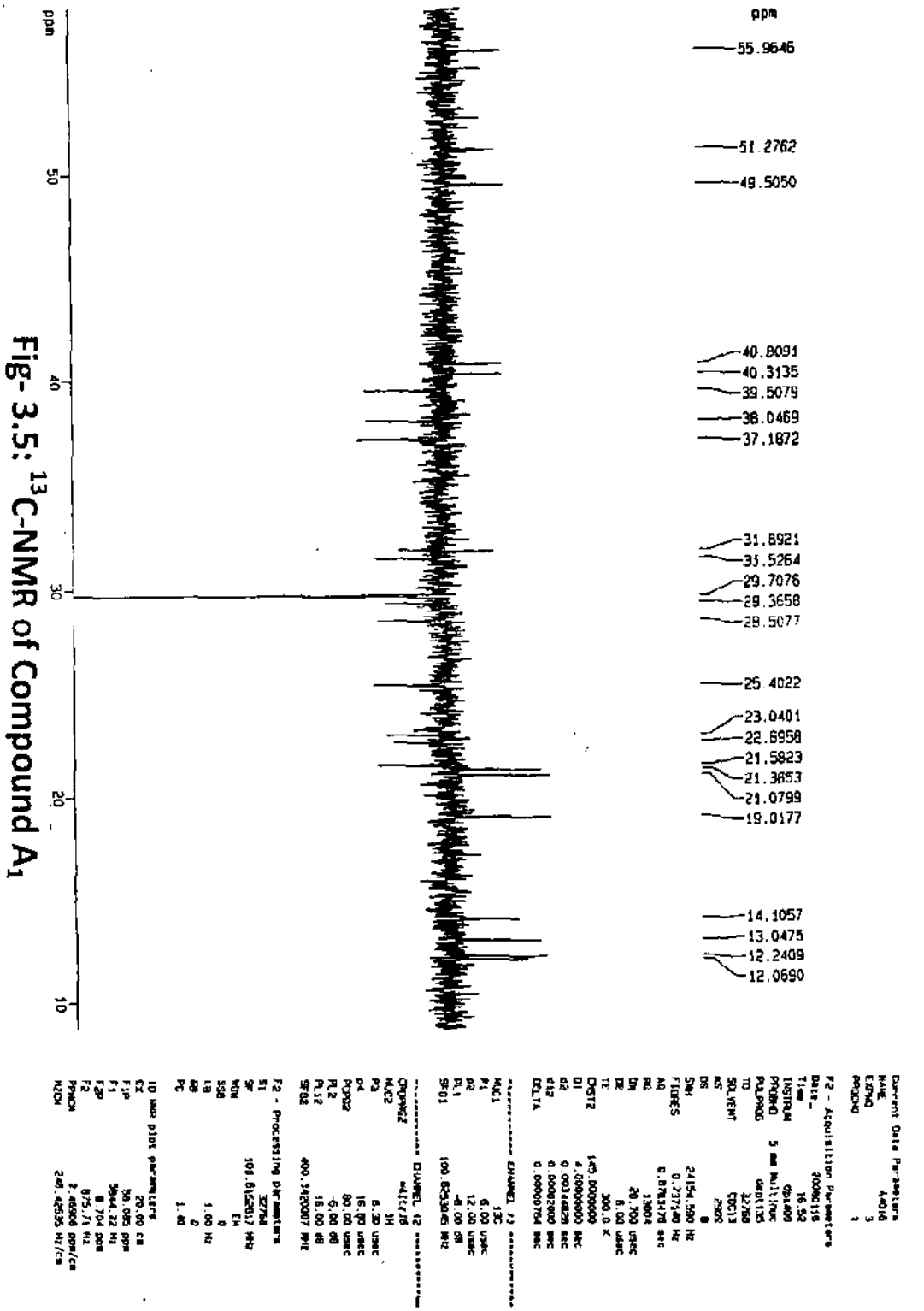


Fig- 3.5: ^{13}C-NMR of Compound A_1

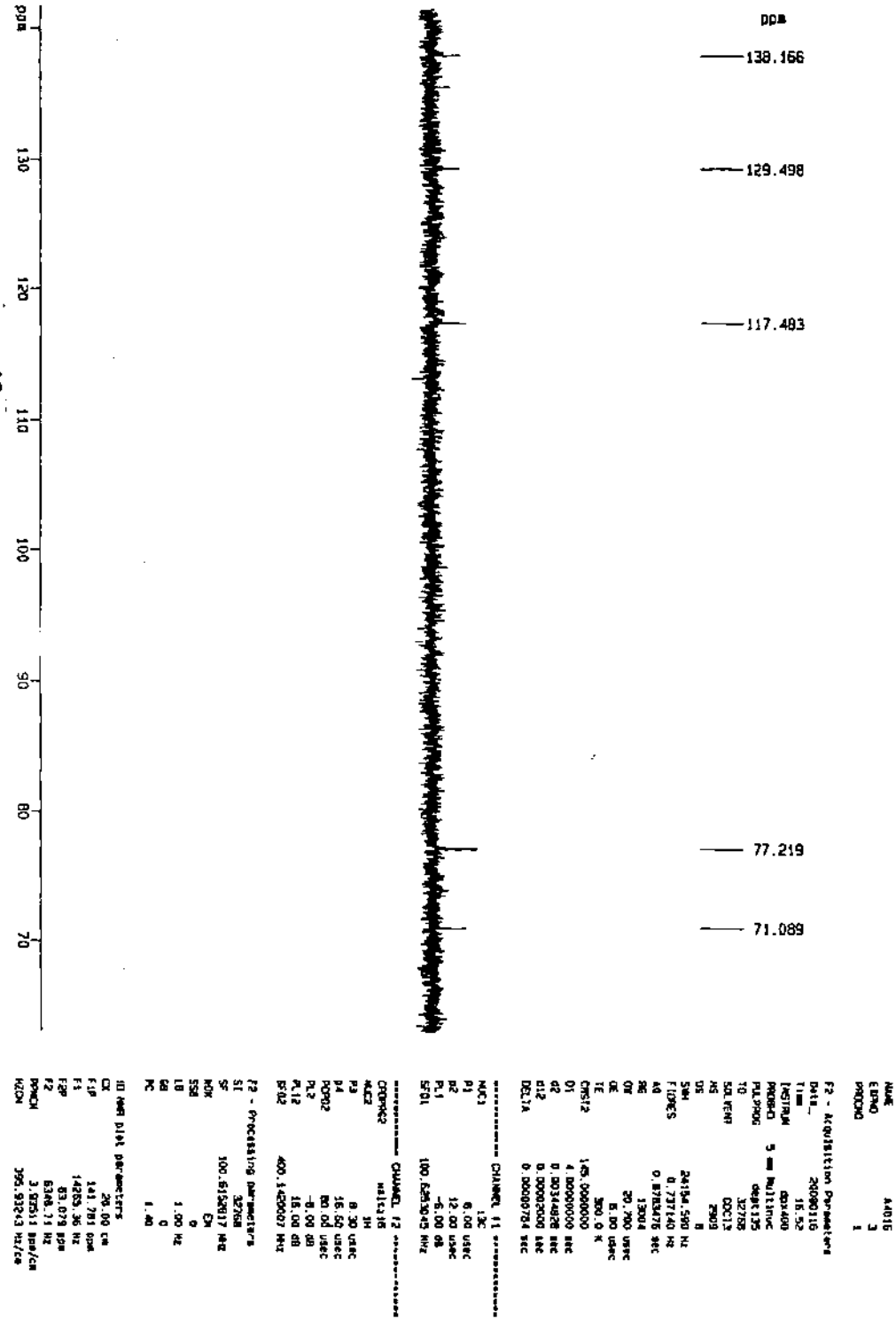


Fig- 3.6: ^{13}C-NMR of Compound A_1

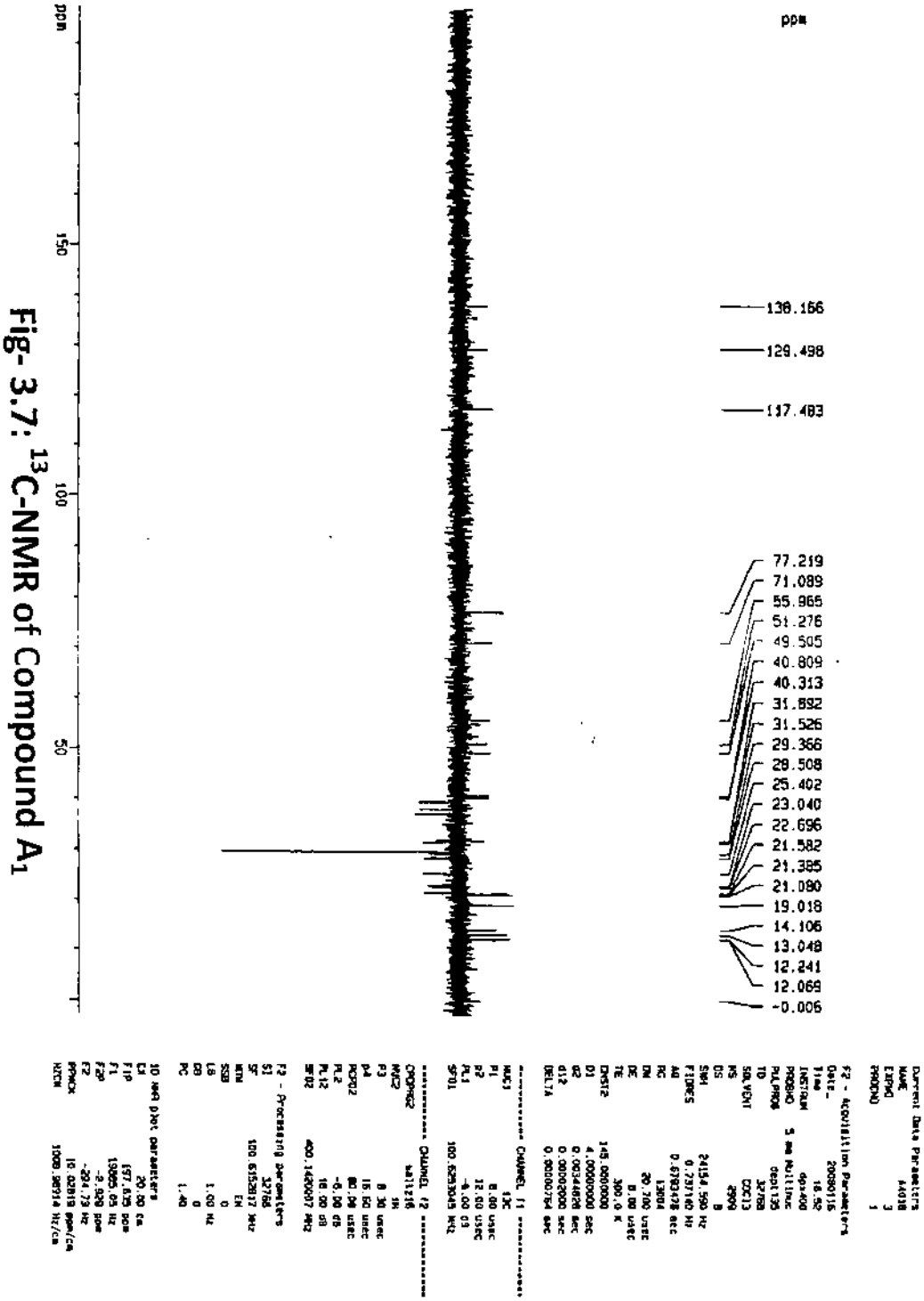


Fig- 3.7: ^{13}C-NMR of Compound A_1

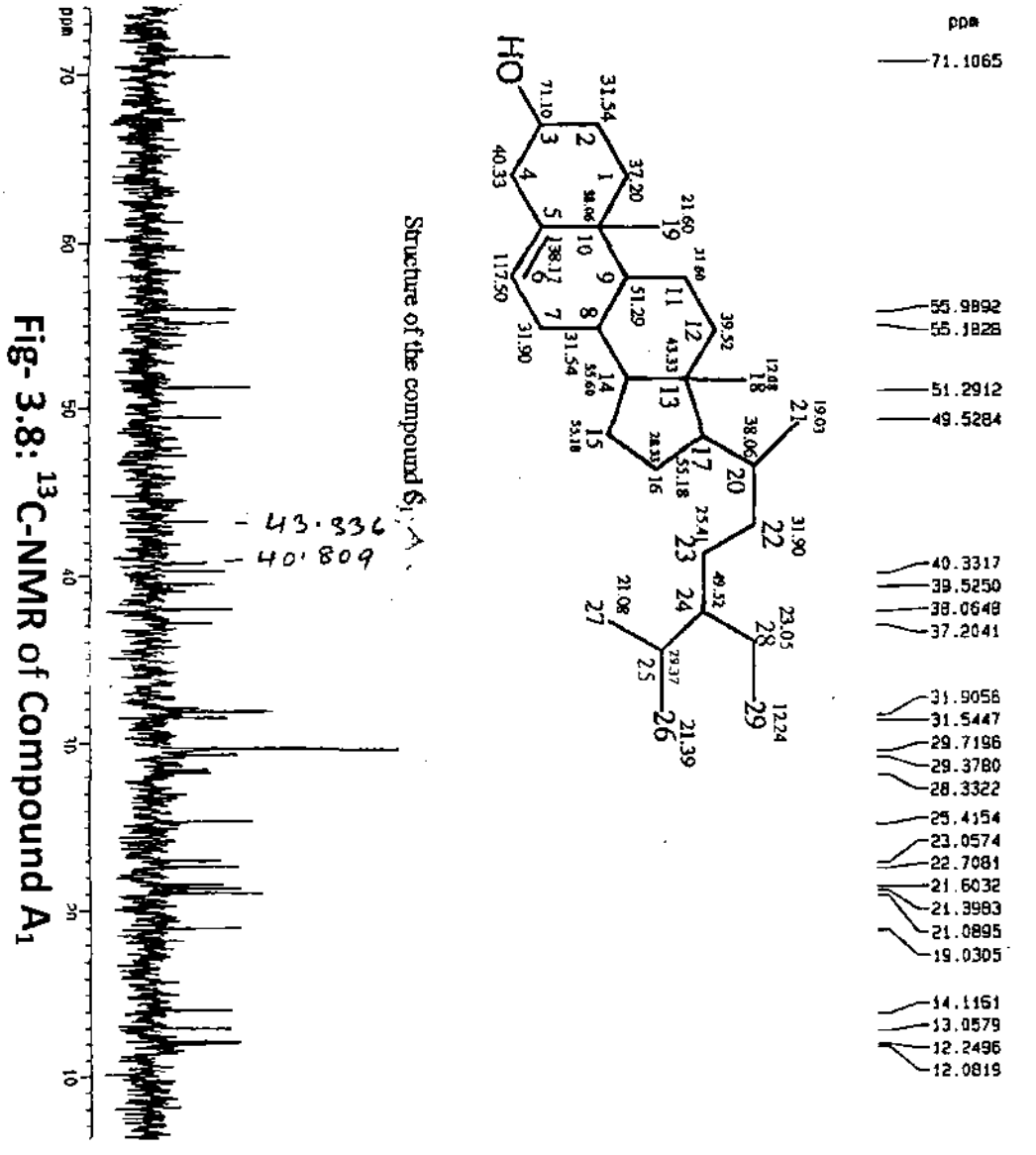


Fig- 3.8: ^{13}C-NMR of Compound A_1

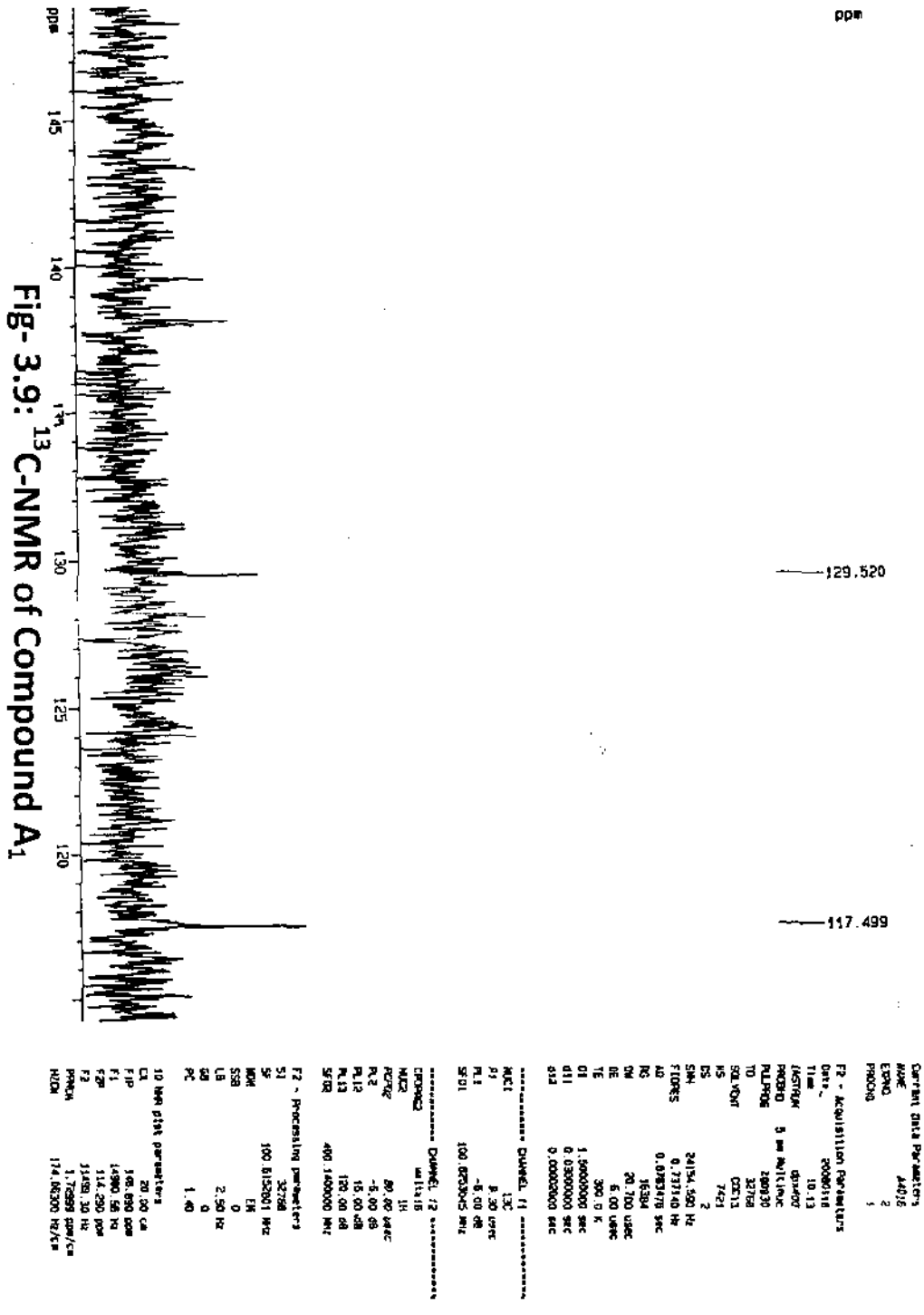


Fig- 3.9: ^{13}C-NMR of Compound A_1

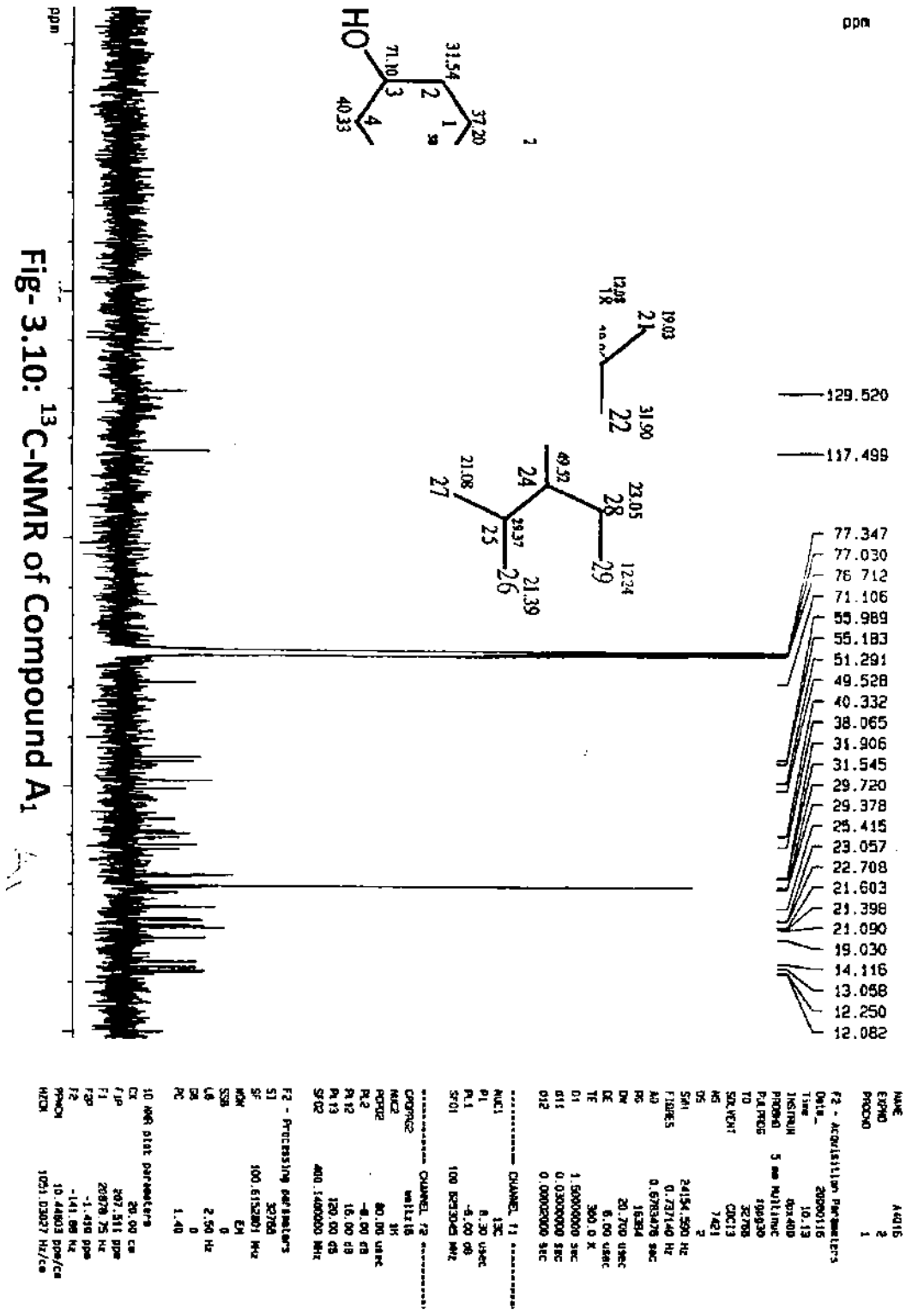
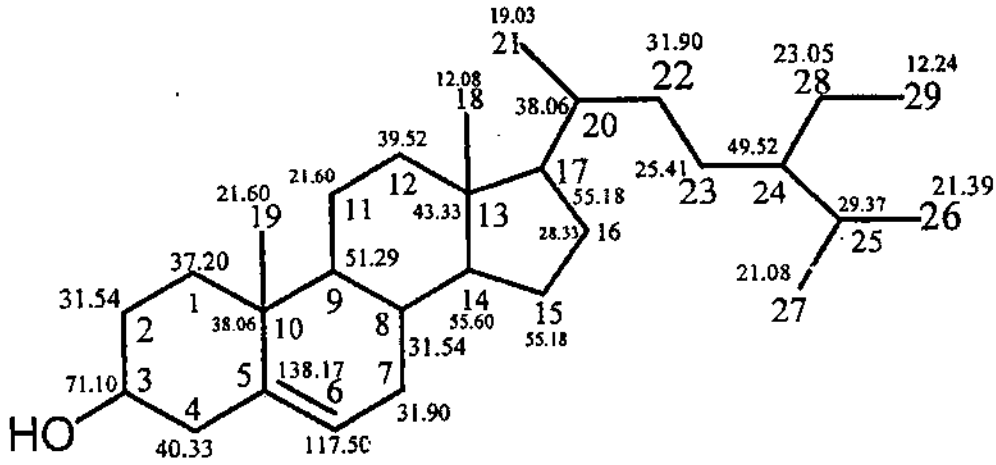


Fig- 3.10: ^{13}C-NMR of Compound A_1

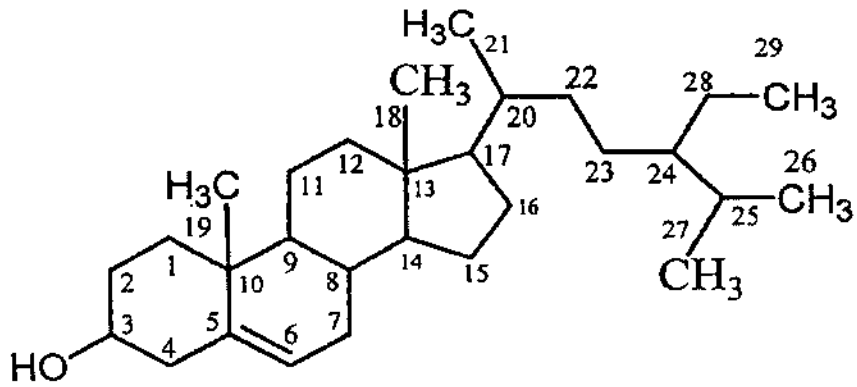
Table 3.2: Comparative ^{13}C-NMR spectral data of the isolated compound A_1 with the published data of β-sitosterol and also with a reported value of β-sitosterol[37].

Carbon No.	Types of carbon	Chemical shift in ppm	
		Observed value for A_1	Reported value[37]
	$-CH_2-$	37.20	37.30
	$-CH_2-$	31.54	31.60
	>CH-OH	71.10	71.70
	$-CH_2-$	40.33	42.30
	>C<	138.17	140.80
	$-CH_2-$	117.50	121.60
	$-CH_2-$	31.90	31.90
	>CH-	31.54	31.90
	>CH-	51.29	50.20
	>C<	38.06	36.50
	$-CH_2-$	21.60	21.10
	$-CH_2-$	39.52	39.80
	>C<	43.33	42.30
	>CH-	55.60	56.80
	$-CH_2-$	55.18	24.30
	$-CH_2-$	28.33	28.30
	>CH-	55.18	56.10
	$-CH_3$	12.08	11.90
	$-CH_3$	21.60	19.40
	>CH-	38.06	36.20
	$-CH_3$	19.03	18.80
	$-CH_2-$	31.90	33.90
	$-CH_2-$	25.41	26.10
	>CH-	49.52	45.90
	>CH-	29.37	29.20
	$-CH_3$	21.39	19.80
	$-CH_3$	21.08	19.10
	$-CH_2-$	23.05	23.10
	$-CH_3$	12.24	12.30

For clarity, all the values were depicted against each carbon in the following structure.



Based on the analysis of IR, ^{1}H and ^{13}C NMR spectroscopic data and comparing the published data of ^{1}H and ^{13}C NMR for β-sitosterol, the compound A_1 was identified as β-sitosterol having the structure as given above. β-sitosterol is a very common compound in plant, used in this report the compound was isolated and identified from the root and stem of the plant *amaranthus sponosus*.



Structure of compound A_1

Although this compound stigmast -5-en-3-β-ol (β-sitosterol) is a known compound, but in this investitgation it was isolated from the chloroform extract of stem and root of the plant *amaranthus spinosus.*

The compound A_2 was white crystalline compound having M.P 203-205^{0}C and its IR spectrum (fig: 3.11) indicated the presence of -OH, - CH_3, - CH_2 and C = C in its structure.

On the other hand compound A_3 was white crystalline with m.p. 175- 180^{0}C and the presence of - OH, -CH_2- and C- O group were indicated by IR spectrum (fig: 3.12).

The other spectral analysis (^{1}H-NMR & ^{13}C-NMR) of compounds A_2 and A_3 were not possible due to their insufficient amount.

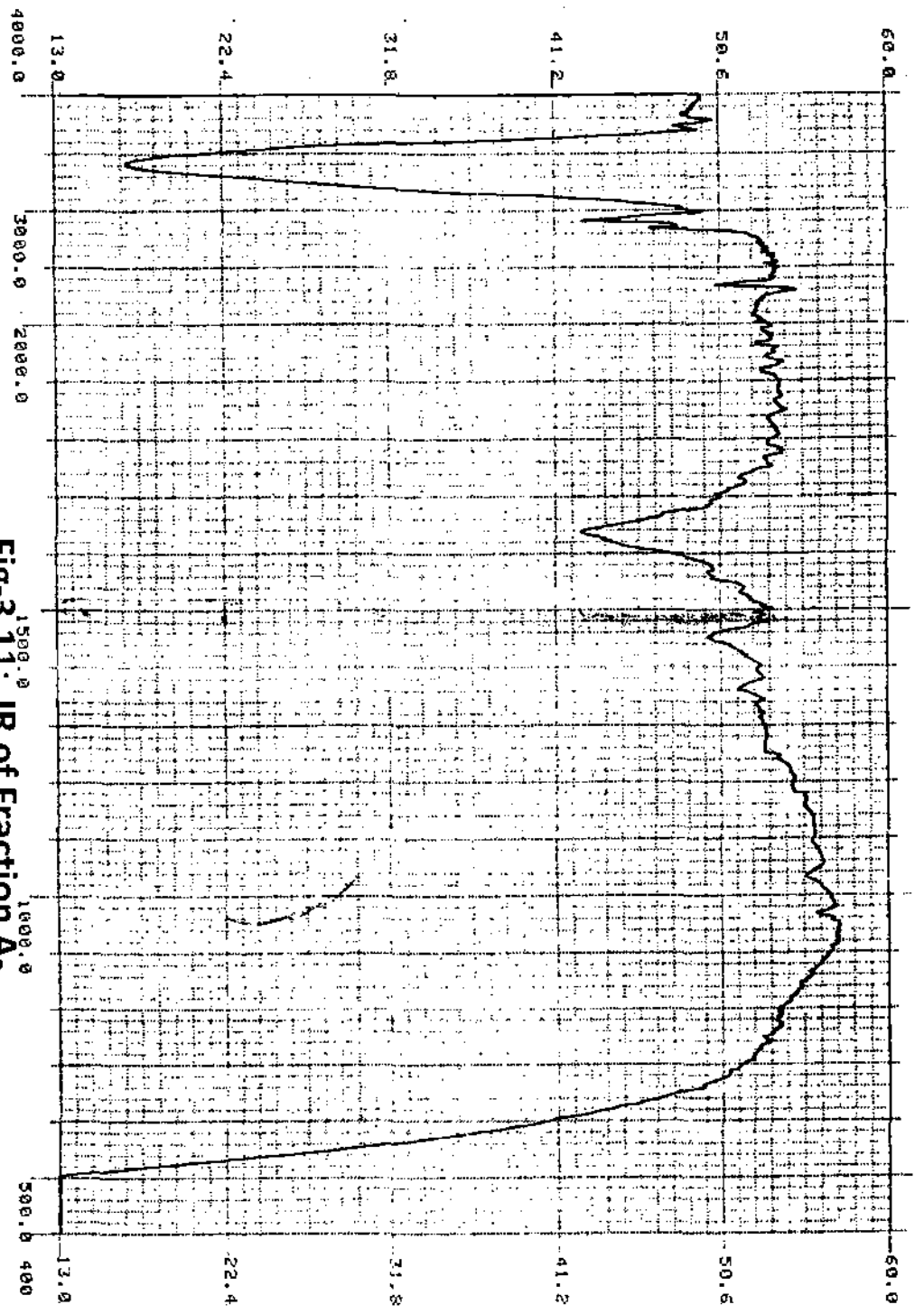


Fig-3.11: IR of Fraction A_2

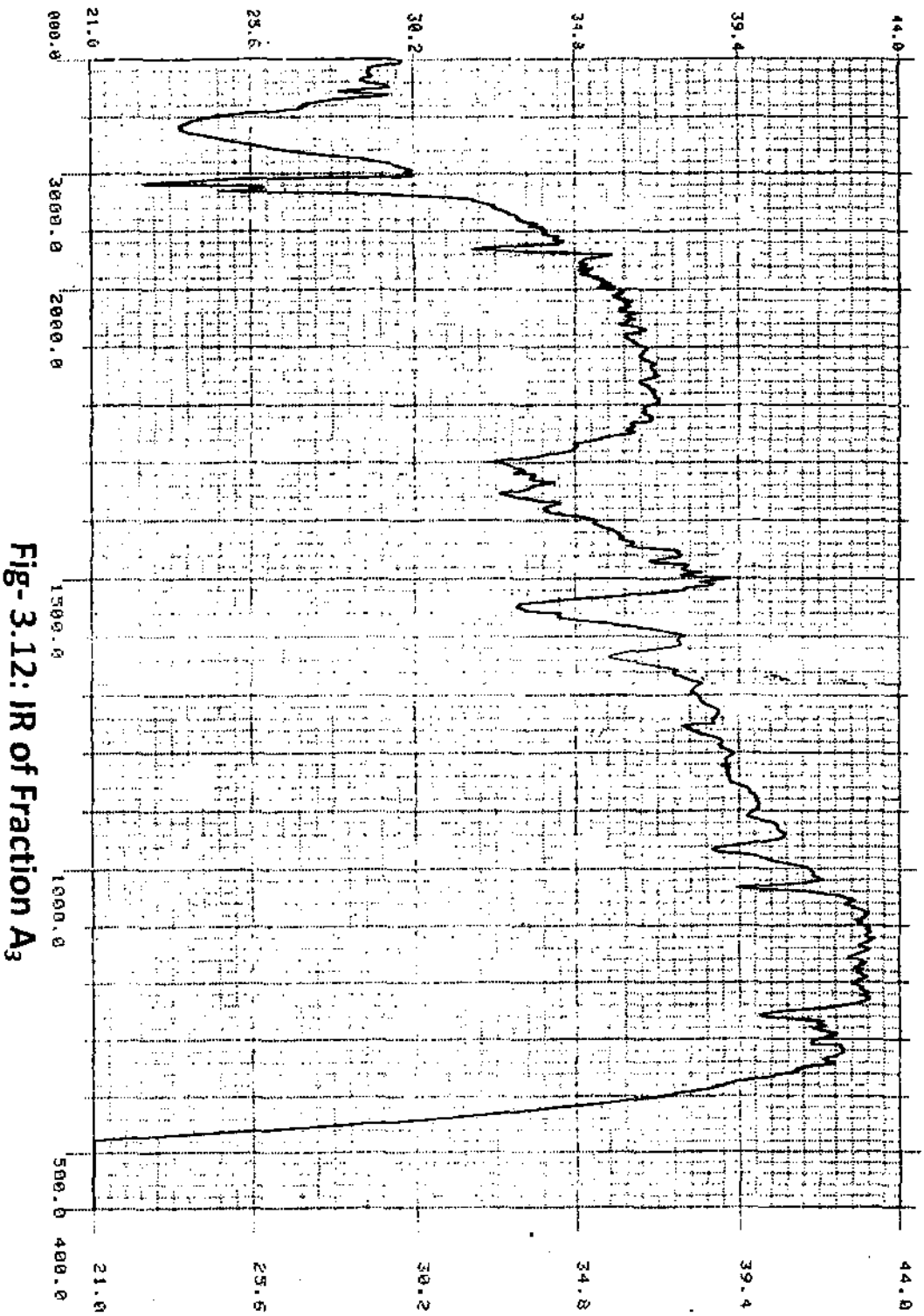


Fig- 3.12: IR of Fraction A_3

3.5.1 CHARACTERISATION OF ISOLATED COMPOUND V_1 AS SPINASTEROL

The compound V_1 was isolated from petroleum ether extract of leaves of the plant as white needle like crystals having melting point 152-54^{0}C. The TLC examination of this isolated compound (pet-ether: ethyl-acetate =3:1) showed brown colored single spot in the iodine chamber. The spot turned into violet on spraying with vanillin-sulfuric acid followed by heating in an oven at 110^{0}C for about 10 minutes. The petroleum ether solution of the isolated compound (V_1) showed the positive test[41] for steroids.

IR spectroscopy:

The analysis of IR spectrum of compound V_1 (Fig-3.13) exhibited a broad absorption band at 3420 cm^{-1} indicative the presence of a hydroxyl, -OH group.

The tiny absorption peak at 3050 cm^{-1} was for H-C=C< skeleton[34]. The two absorption peaks at 2930 cm^{-1} and 2850 cm^{-1} were due to the presence of aliphatic C-H stretching of either both $-CH_3$, $-CH_2$-or - CH< group.

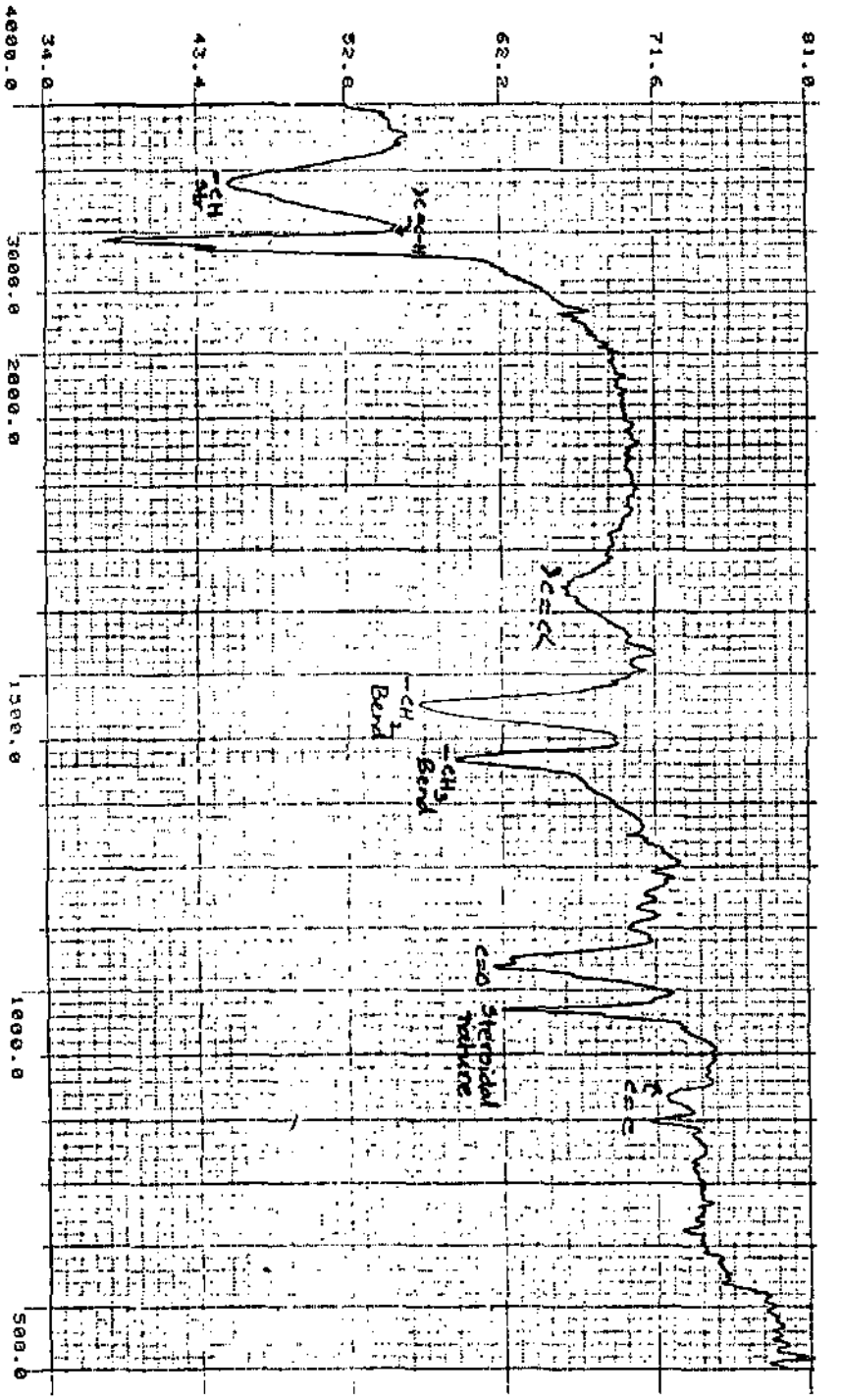


Figure- 3.13: IR spectrum of compound V_1

The peak at 1640 cm^{-1} was suggestive of a >C=C< double bond skeletal unit. The two absorption bands at 1450 cm^{-1} and 1370 cm^{-1} were demonstrative of C-H bending of methylene ($-CH_2-$) and methyl ($-CH_3$) group respectively.

The absorption band at 1040 cm^{-1} was due to C-O stretching for secondary alcoholic group. The sharp band at 970 cm^{-1} was for steroidal nature [41] of the isolated compound. The two peaks at 820 cm^{-1} and 840 cm^{-1} were also due to the presence of a C=C double bond.

^{1}H-NMR spectroscopy:

The ^{1}H-NMR (400 MHz, in $CDCl_3$) spectrum (Fig-3.14 & 3.15) of the isolated compound V_1 had a sharp singlet at 0.540 ppm indicative of the three protons of methyl group at C-13. The singlet at 0.812 ppm was indicative of the presence of three protons of the methyl group at C-10.

The spectrum showed a doublet at 1.024 ppm with coupling constant, J=6.67 Hz was due to the presence of methyl protons at C-20, a broad singlet at 0.794 ppm was due to the presence of methyl protons (27-H3) at C-25 and a doublet at 0.847 ppm (J=5.83) was due to the presence of methyl protons (26-H3) at C-25. The triplet underneath at 0.795 ppm was due to the presence of methyl protons (29-H3) at C-28 and the multiplate at δ 3.584 ppm was due to the presence of a methine proton (3α-H) at C-3 of a steroidal nucleus. The spectrum showed a multiplate as characteristics downfield signals at δ 5.12-5.14 ppm was due to the presence of olefinic protons 22-H and 23-H at C-22 and C-23 and a multiplate at5.04 ppm was due to the presence of a proton (7-H) at C-7 of a steroidal nucleus. The multiplate between 1.40-2.00 ppm was due to the presence of methane and methylene protons and a singlet at δ 1.009 ppm was suggestive the presence of a proton (3-OH) of hydroxyl group at C-3.

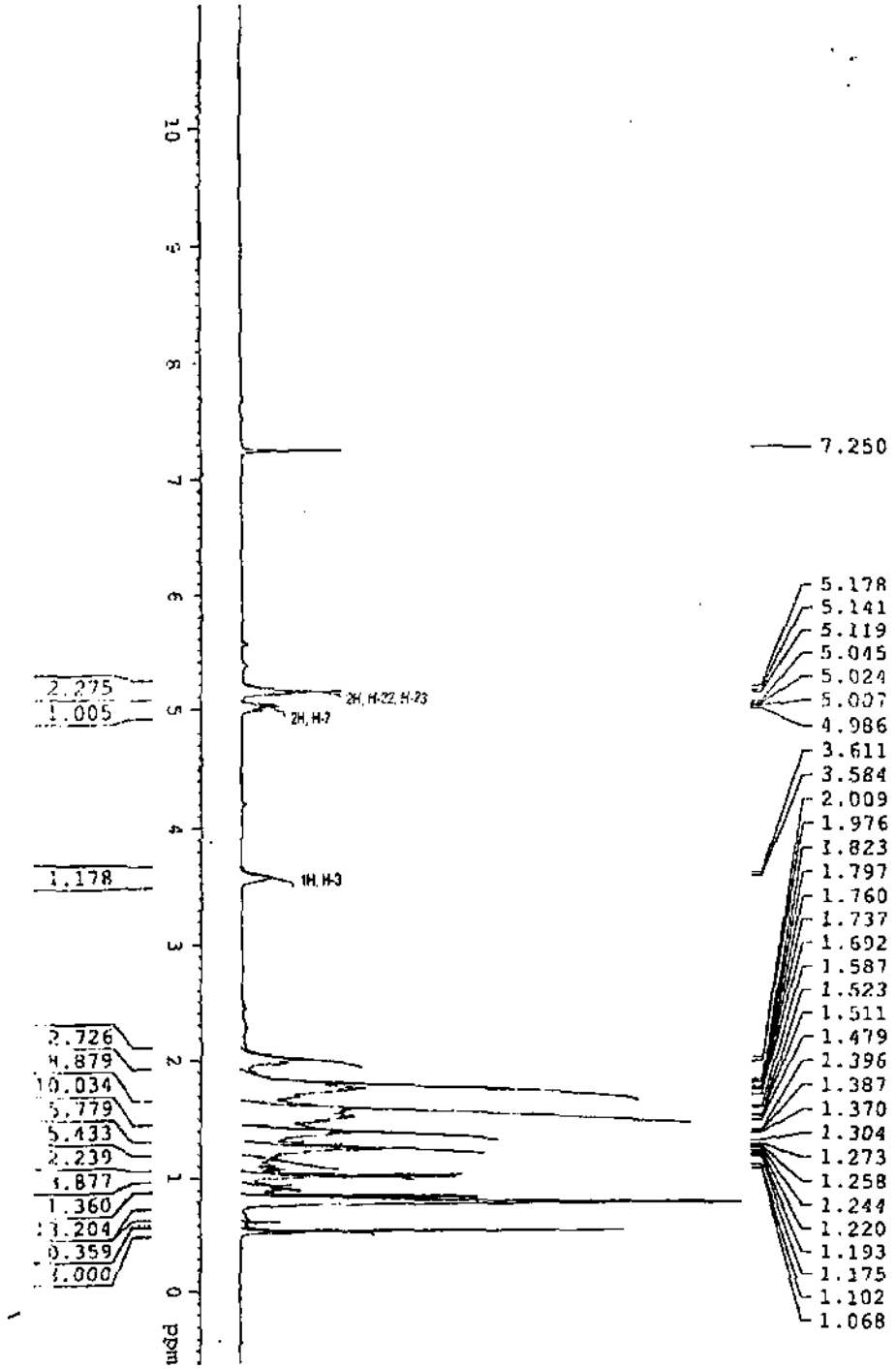


Figure- 3.14: ^{1}H-NMR spectrum of compound V_1

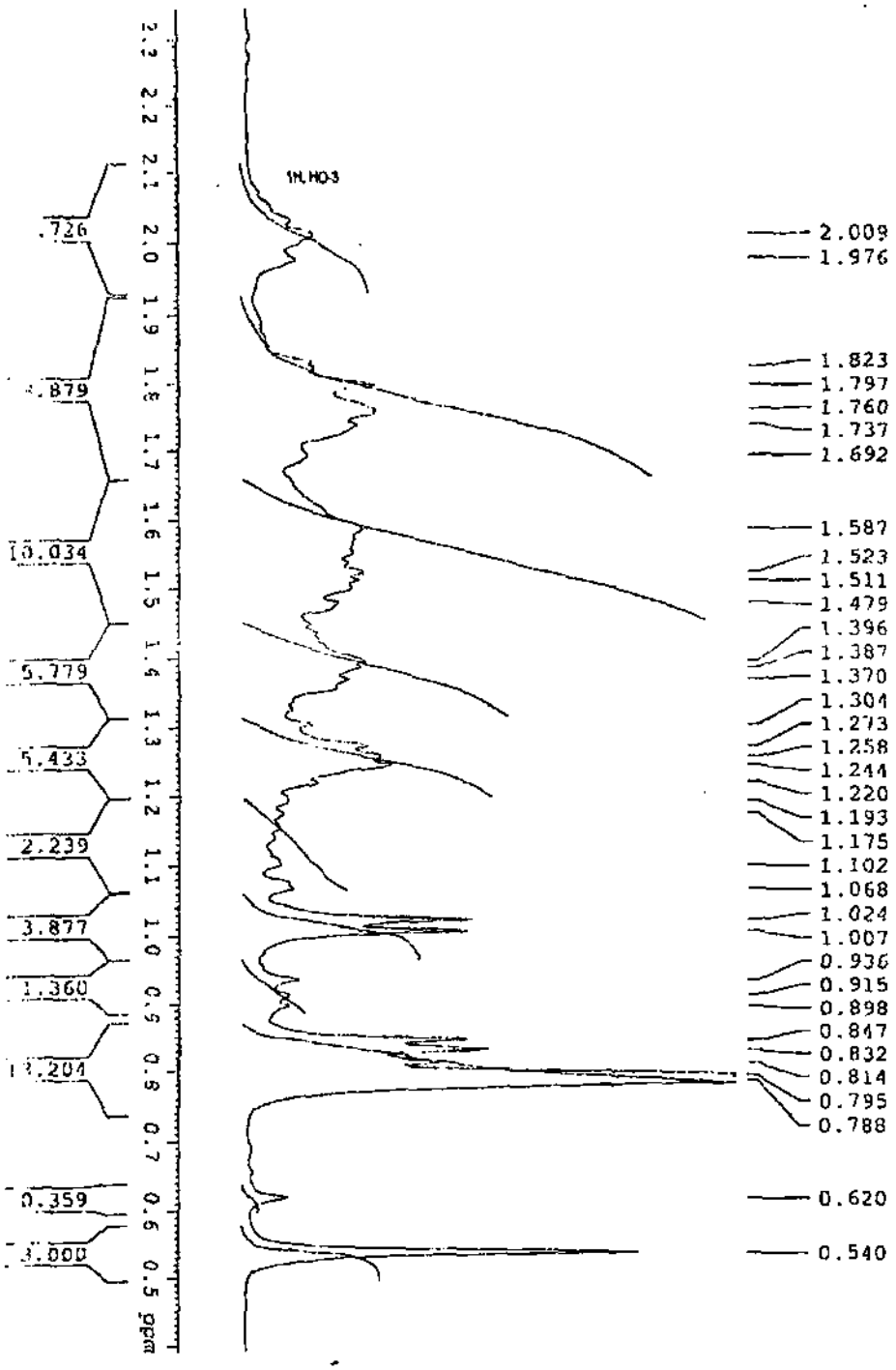


Figure- 3.15: ^{1}H-NMR spectrum of compound V_1

Table 3.3

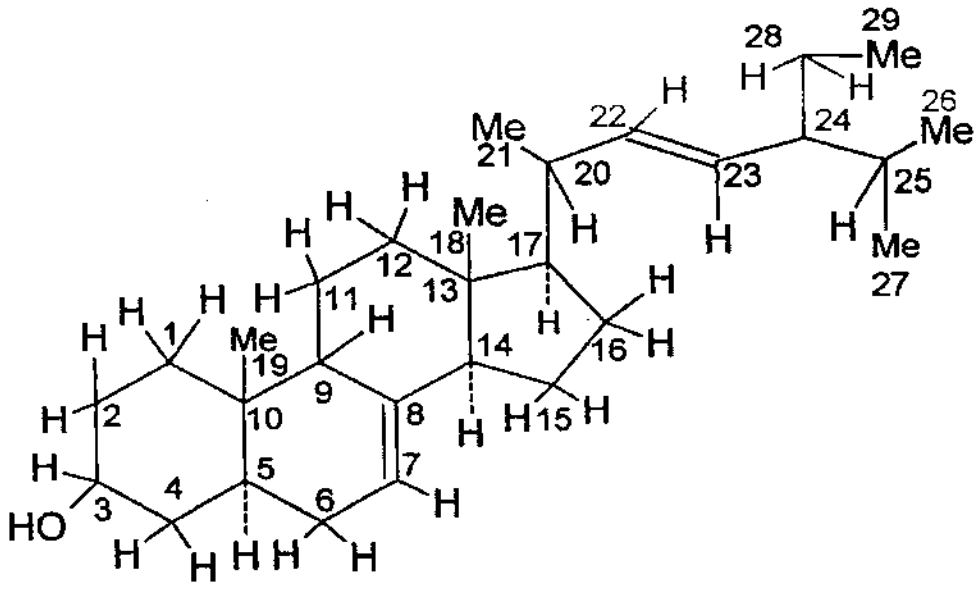
Comparative ^{1}H-NMR spectral data of isolated compound V_1 with the published data[39] of spinastaryl acetate.

Protons	δ value of the isolated compound V_1 in ppm	Published[33] δ value in ppm
3α-H	δ 3.584 m	δ 4.5 m
3-OH	δ 2.009 s	δ 2.023 s (acetyl)
7-H	δ 5.024 m	δ 5.13 m
22-H & 23-H	δ 5.12-5.14 m	δ 5.10-5.15 m
18-H_3	δ 0.540 s	δ 0.544 s
19-H_3	δ 0.814 s	δ 0.819 s
21-H_3	δ 1.024 d, J=6.67 Hz	δ 1.030 d, J=6.5 Hz
	δ 0.847 d, J=5.9 Hz	δ 0.853 d, J=6.8 Hz
26-H_3	δ 0.795 broad s	δ 0.805 d, J=6.8 Hz
27-H_3	underneath at 0.795	δ 0.808 t, J=7.2 Hz
29-H_3	δ 1.40 - 2.0 m, t, d, s	-
Methine and methylene protons		

^{13}C-NMR spectroscopy:

The ^{13}C-NMR spectrum (Fig-3.16, 3.17, 3.18 & 3.19) of isolated compound, V_1 showed the presence of twenty-nine (29) carbon signals. Among these 29 carbon signals, four (4) of them were assignable to sp^2 carbons, nine (9) signals to methylene carbons, six (6) signals to methyl carbons, eight (8) signals to methane carbons and two (2) signals for quaternary carbons.

The signals at δ_c 21.41, 23.11, 25.41, 28.52, 29.67, 31.52, 37.18, 38.03 and 39.51 ppm were due to nine (9) methylene carbons, signals at δ_c 32.01, 40.30, 40.84, 49.49, 51.28, 55.21, 55.94 and 71.09 ppm were due to eight (8) methine carbons.



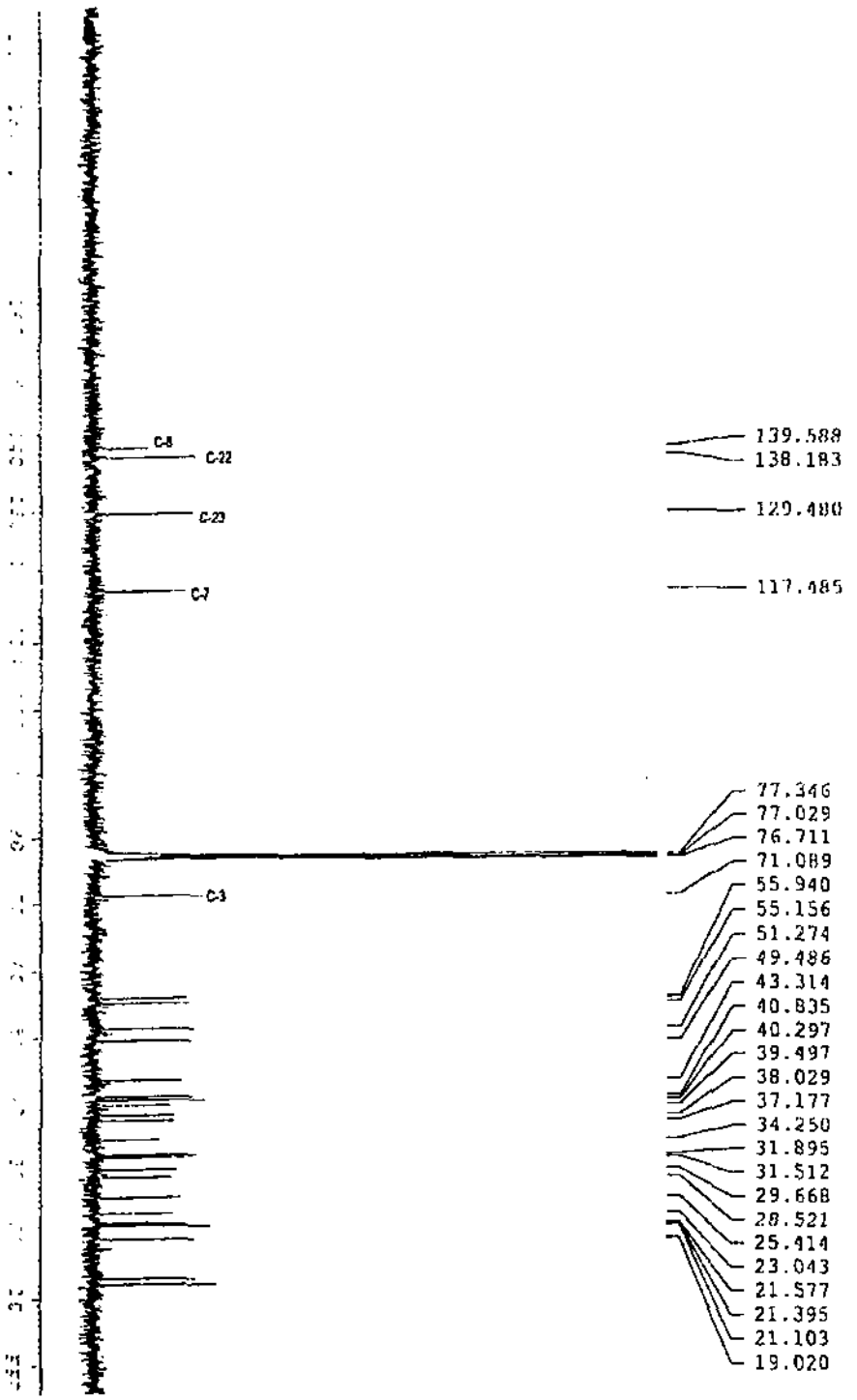


Figure- 3.16: ^{13}C-NMR spectrum of compound V_1

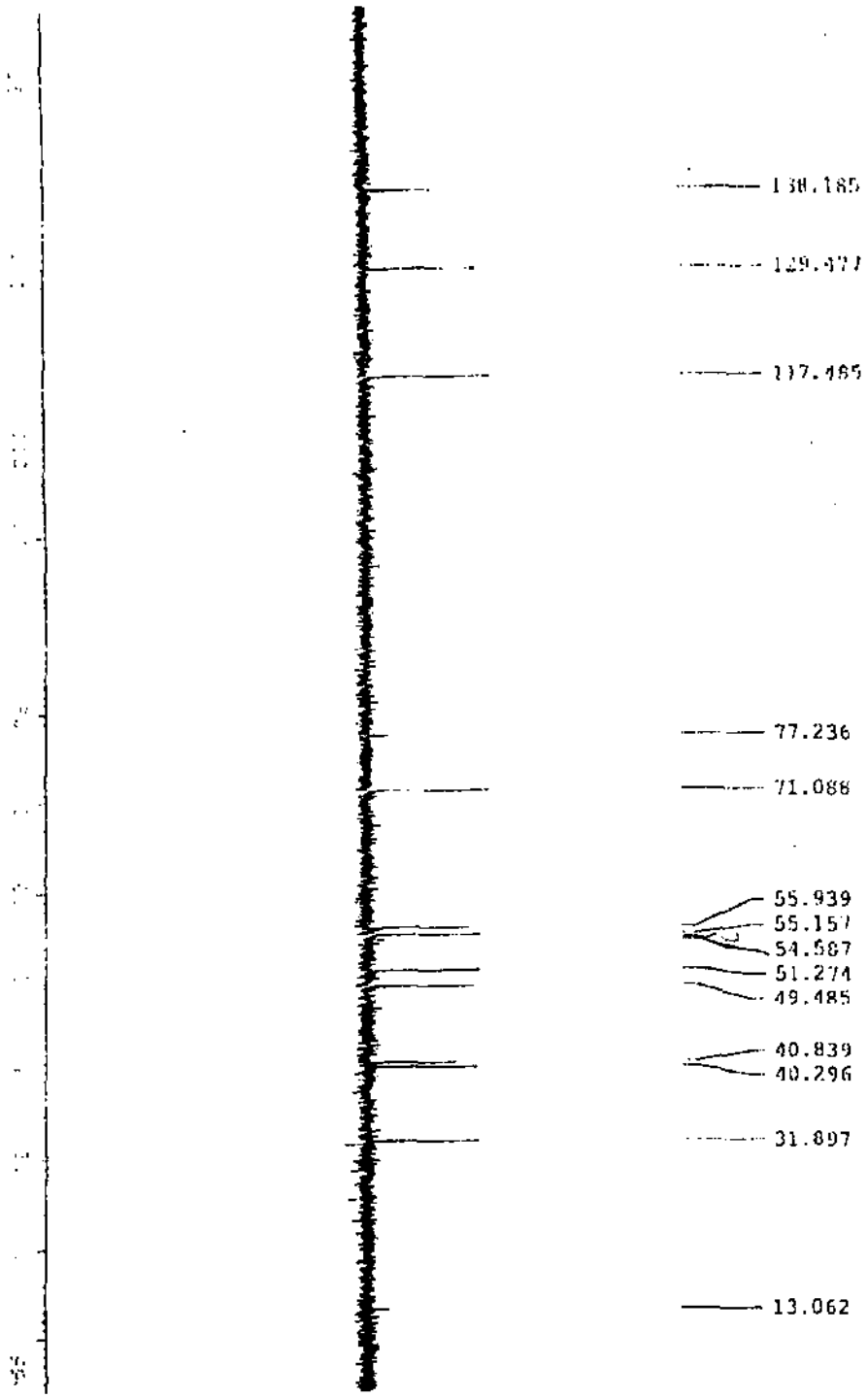


Figure- 3.17: Expended ^{13}C-NMR spectrum of compound V_1

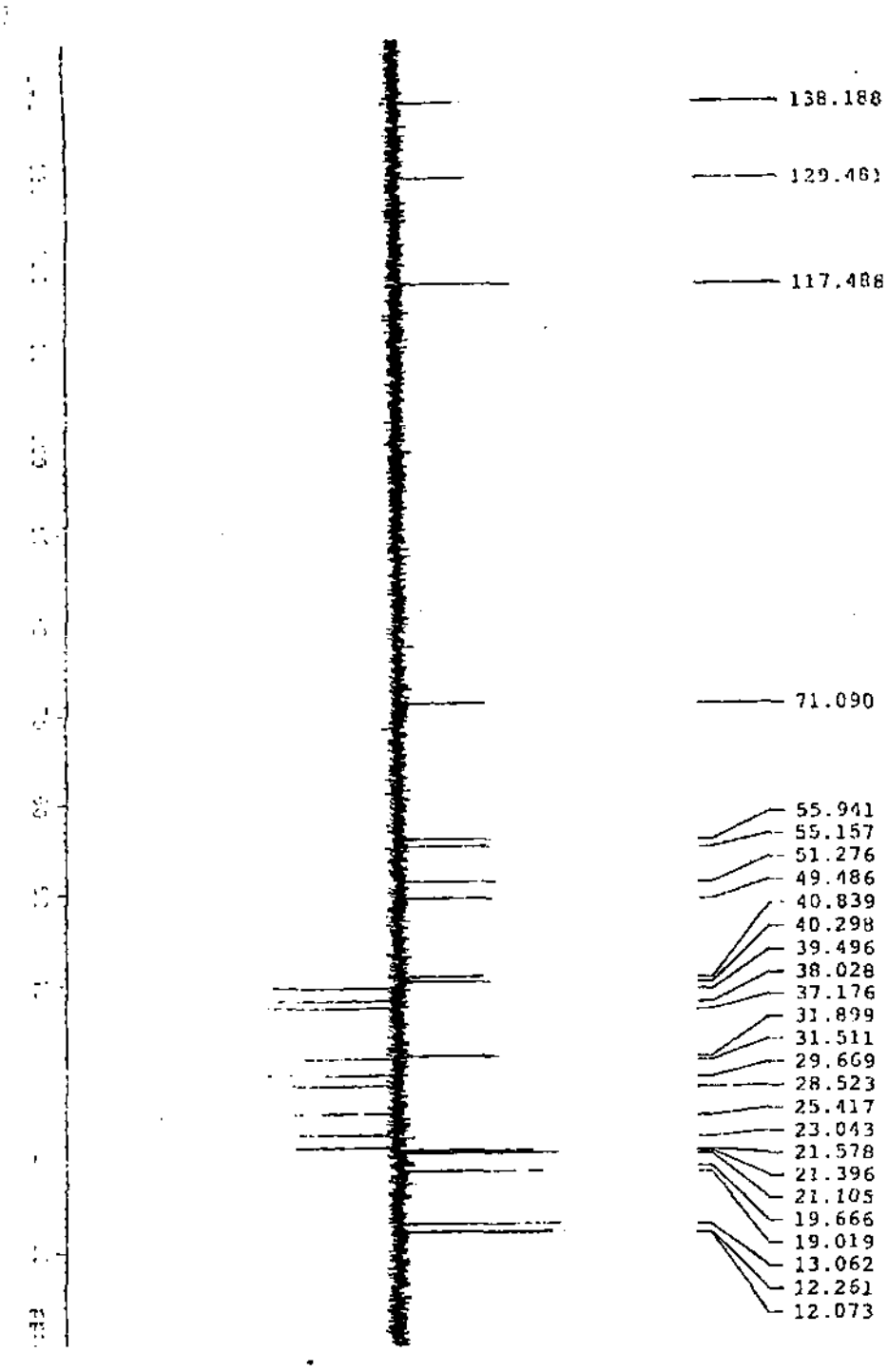


Figure- 3.18: Dept 135 spectrum of compound V_1

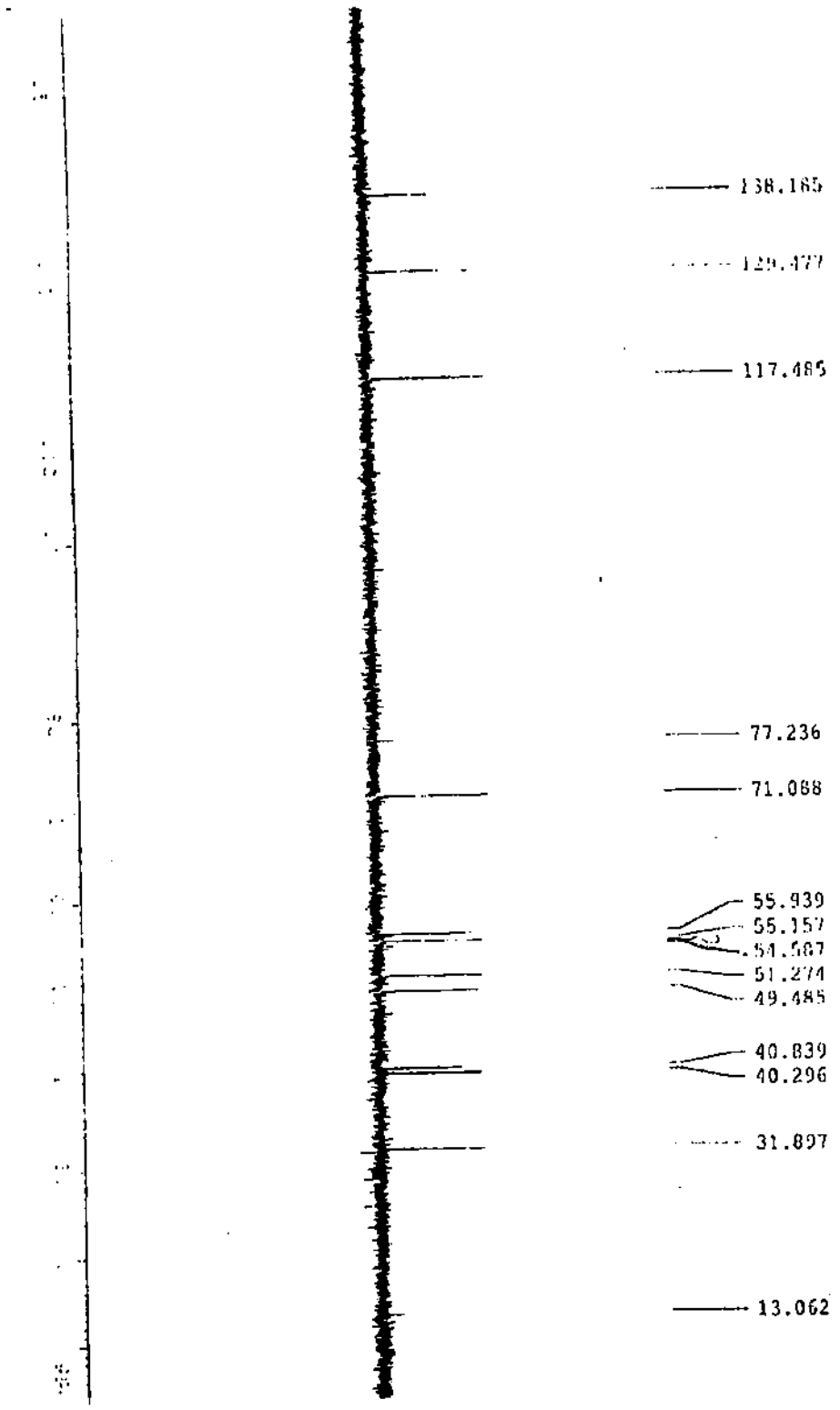
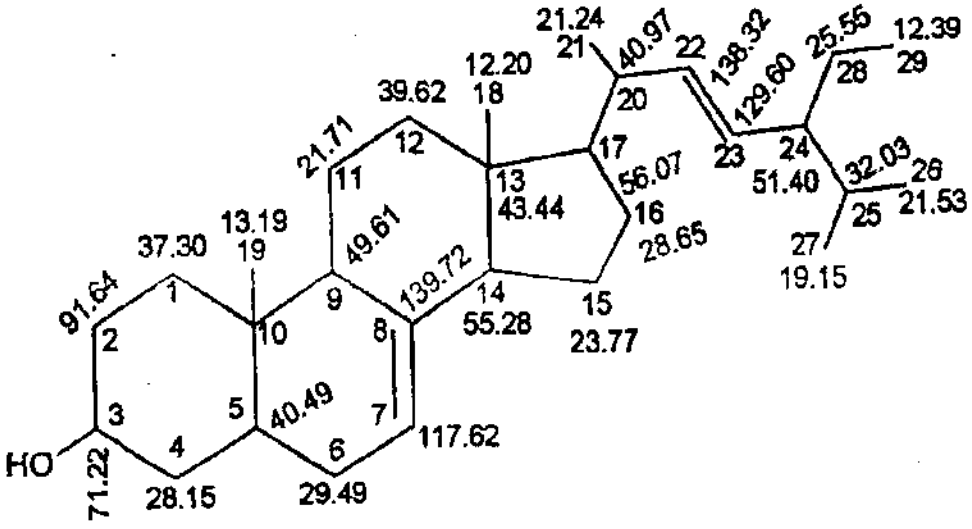


Figure- 3.19: Dept 90 speetrum of compound V_1

The signals appeared at δ_c 12.15, 12.31, 13.11, 19.02, 21.39 and 21.41 were due to the six methyl carbons and the signals appeared at δ_c 117.49, 129.48, 138.18 and 139.59 due to four olefinic carbons. The quaternary two (2) carbons resonated at δc 34.30 and 43.32 ppm and the signal at 71.09 ppm was due to methine hydroxy (>HC-OH) carbon.

For clarity, all the δ_c values were depicted against each carbon in the following structure.



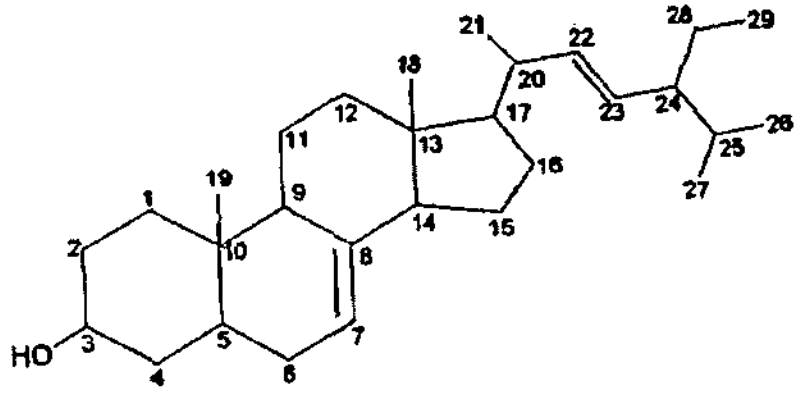
Structure of Compound V_1

The ^{13}C-NMR data of the compound V_1 was compared with the published ^{13}C-NMR data of spinasterol[40] (Table-3.4).

Table-3.4: Comparative ^{13}C-NMR spectral data of the isolated compound V_1 and spinasterol[40].

Carbon Number	Types of carbon	Chemical shift in ppm	
		Isolated compound V_1	Spinasterol[40]
1	- CH_2 -	37.18	37.2
2	- CH_2 -	31.52	31.6
3	H–C–O–H	71.09	71.1
4	- CH_2 -	38.03	38.1
5	>CH–	40.30	40.4
6	- CH_2 -	29.67	29.7
7	=CH<	117.49	117.5
8	=CH<	139.59	139.6
9	>CH–	49.49	49.6
10	>C<	34.30	34.3
11	- CH_2 -	21.60	21.6
12	- CH_2 -	39.51	39.6
13	>C<	43.32	43.3
14	>CH–	55.21	55.2
15	- CH_2 -	23.11	23.1
16	- CH_2 -	28.52	28.6
17	>CH–	55.94	56.0
18	- CH_3 -	12.15	12.1
19	- CH_3 -	13.11	13.1
20	>CH–	40.84	40.8
21	- CH_3 -	21.39	21.1
22	=CH–	129.48	129.5
23	=CH–	129.48	129.5
24	>CH–	51.28	51.3
25	>CH–	32.01	32.0
26	- CH_3 -	21.41	21.3
27	- CH_3 -	19.02	19.0
28	- CH_2 -	25.41	25.5
29	- CH_3 -	12.31	12.3

Based on the IR, ^{1}H- and ^{13}C-NMR spectroscopic data, the compound V_1 has been proposed to have the following structure.



Structure of Compound V_1

This is a known compound (22E) Stigmast-7, 22-dien-3β-ol (spinasterol). The structure of the compound V_1 was established as (22E) Stignast-7, 22- dien-3β-ol (spinasterol). These are well known compounds in nature. The previous workers[27-31] isolated and characterized the same compounds from the pet ether (b. p 40-60^{0}C) extracts of stems of Katanotay (*Amaranthus spinosus Linn.)* Isolation and characterization of this compound from the leaves of Katanotay (*Amaranthus spinosus Linn.)* was reported here for the first time.

3.5.2 CHARACTERIZATION OF ISOLATED COMPOUND V_2 AS 1-NONADECANOL.

The compound 'V_2' was isolated from the fraction C_9 of petroleum ether extract of the leaves of *Amaranthus spinosus*. Its TLC showed a single compound upon exposure to iodine vapor and also with vanillin-sulfuric acid spray followed by heating in oven. It was obtained as white needle shaped crystals m.p. (61-62^{0}C). It was readily soluble in mixture of solvent (80% Pet-ether: 20% ethyl-acetate) and chloroform. Its IR spectrum (Fig 3.20) showed a broad absorption band at 3450 cm^{-1} assignable to hydrogen bonded hydroxyl group (-OH). The bands at 1430, 1350 together with the bands at 2950 cm^{-1} & 2850 cm^{-1} were due to the presence of -CH_2 and -CH_3 group respectively. The absorption band at 1050 cm^{-1} was due to the presence of C-O stretching for alcoholic group. IR spectrum of V_2 is similar to 1-hexanol[35].

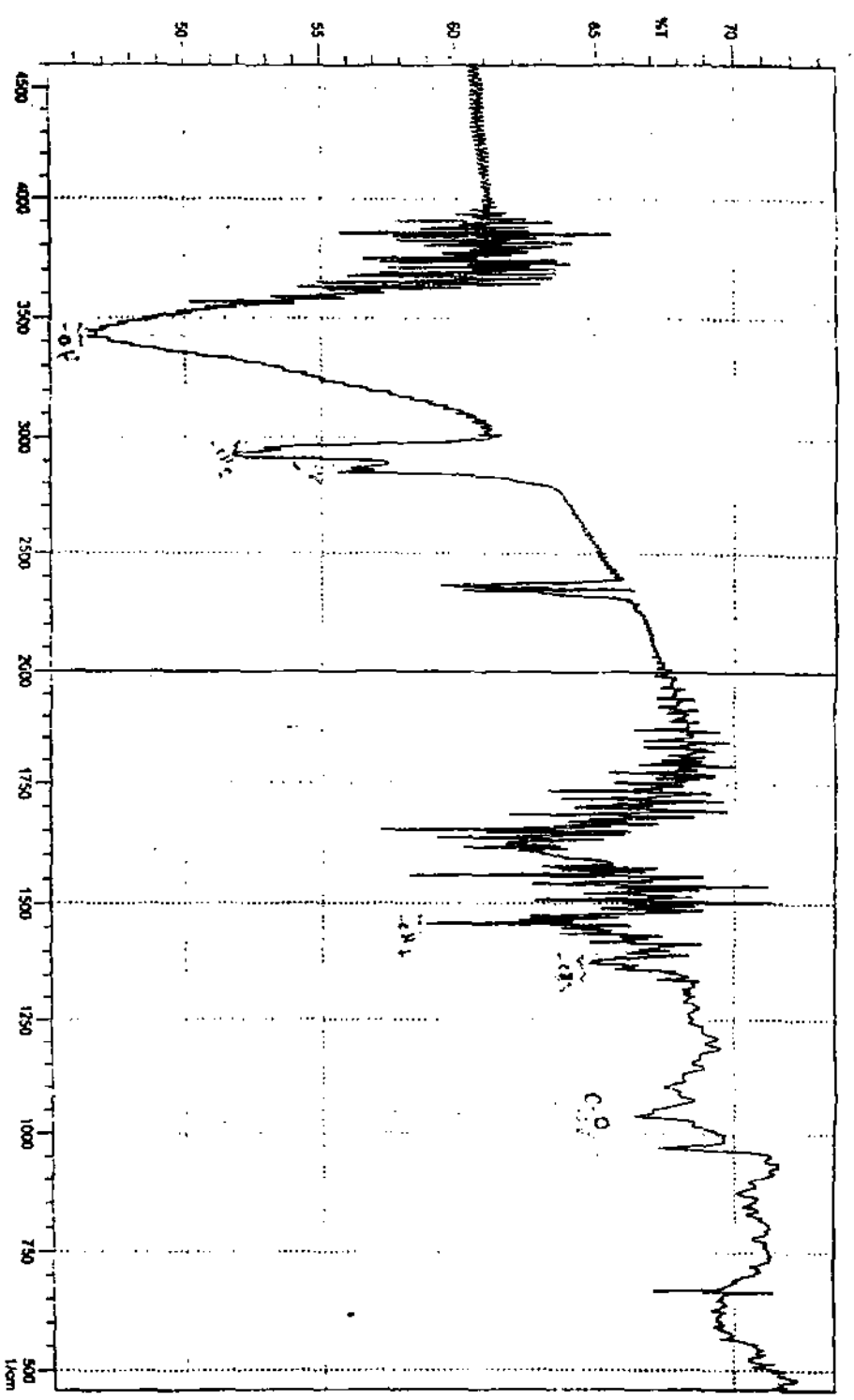


Figure- 3.20: IR spectrum of eompound V_2

The ^{1}H-NMR spectrum (Fig: 3.21 & 3.22) showed a downfield broadened singlet at δ 4.061 ppm suggestive of the presence of hydrogen bonded primary alcoholic proton[9]. Triplet at δ 1.603 ppm is due to methylene protons, which attach to hydroxyl and methylene group (-CH_2-CH_2-OH). The single broadened absorption peak at 1.245 ppm for the protons of seventeen- methylene group, which have similar chemical shift and same coupling constant in long chain[35]. The spectrum showed up field triplet at 0.871 ppm for the methyl group, which attach to methylene group[39].

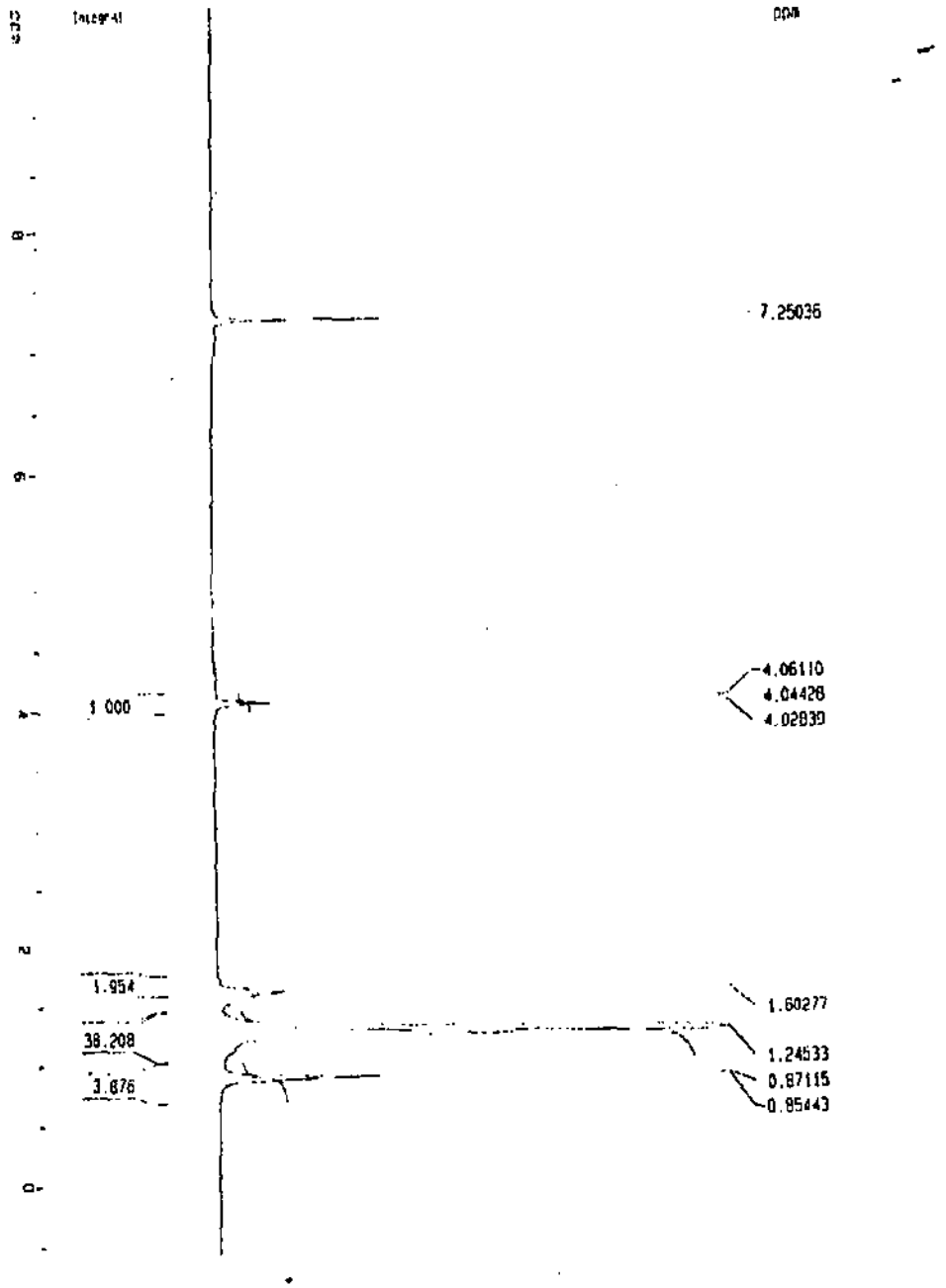


Figure- 3.21: ^{1}H-NMR spectrum of compound V_2

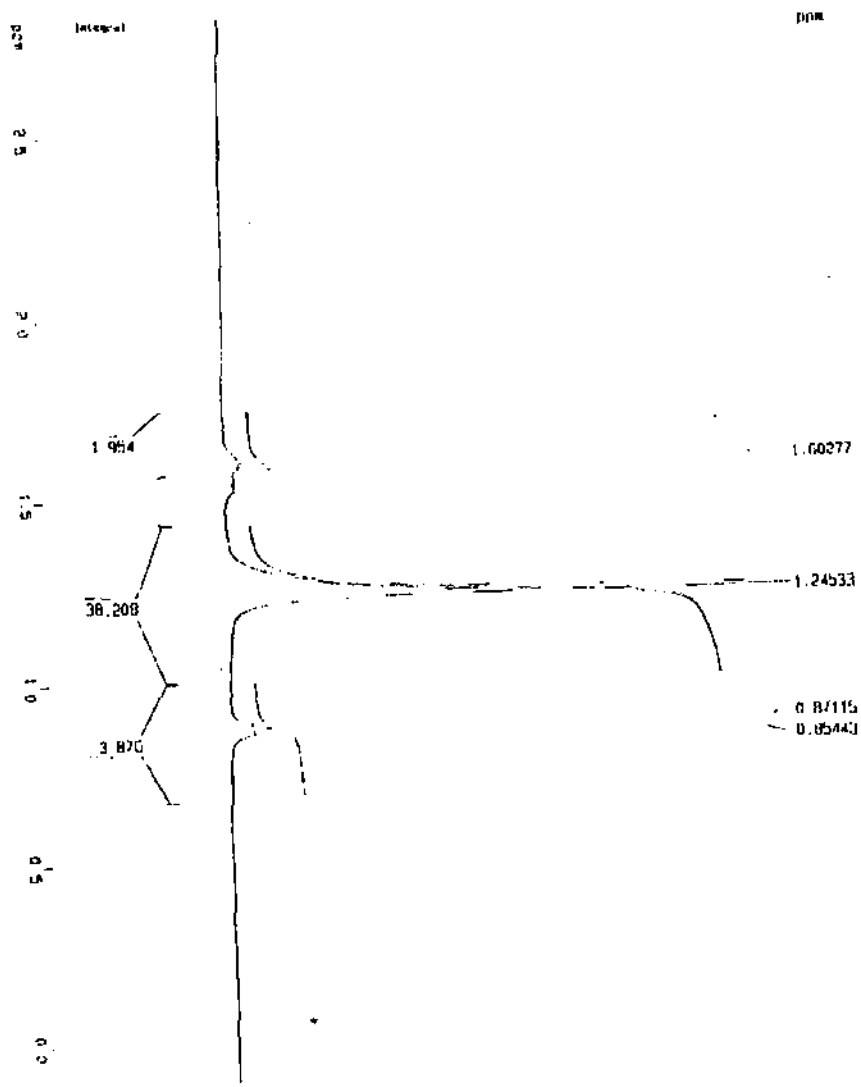


Figure- 3.22: Expended ^{1}H-NMR spectrum of compound V_2

The ^{13}C-NMR spectral analysis of V_2 showed signal at 64.482 was suggestive of a methylene carbon being attached to a hydroxyl group[34]. The upfield signal (Fig: 3.23 & 3.24) at 14.137 indicated the presence of methyl carbon. The signals at 22.719- 34.462 for seventenn- methylene carbon, which were existing in long chain of primary alcohol[34]. by DEPT experiment, methyl and methylene carbons were fixed (Fig: 3.25).

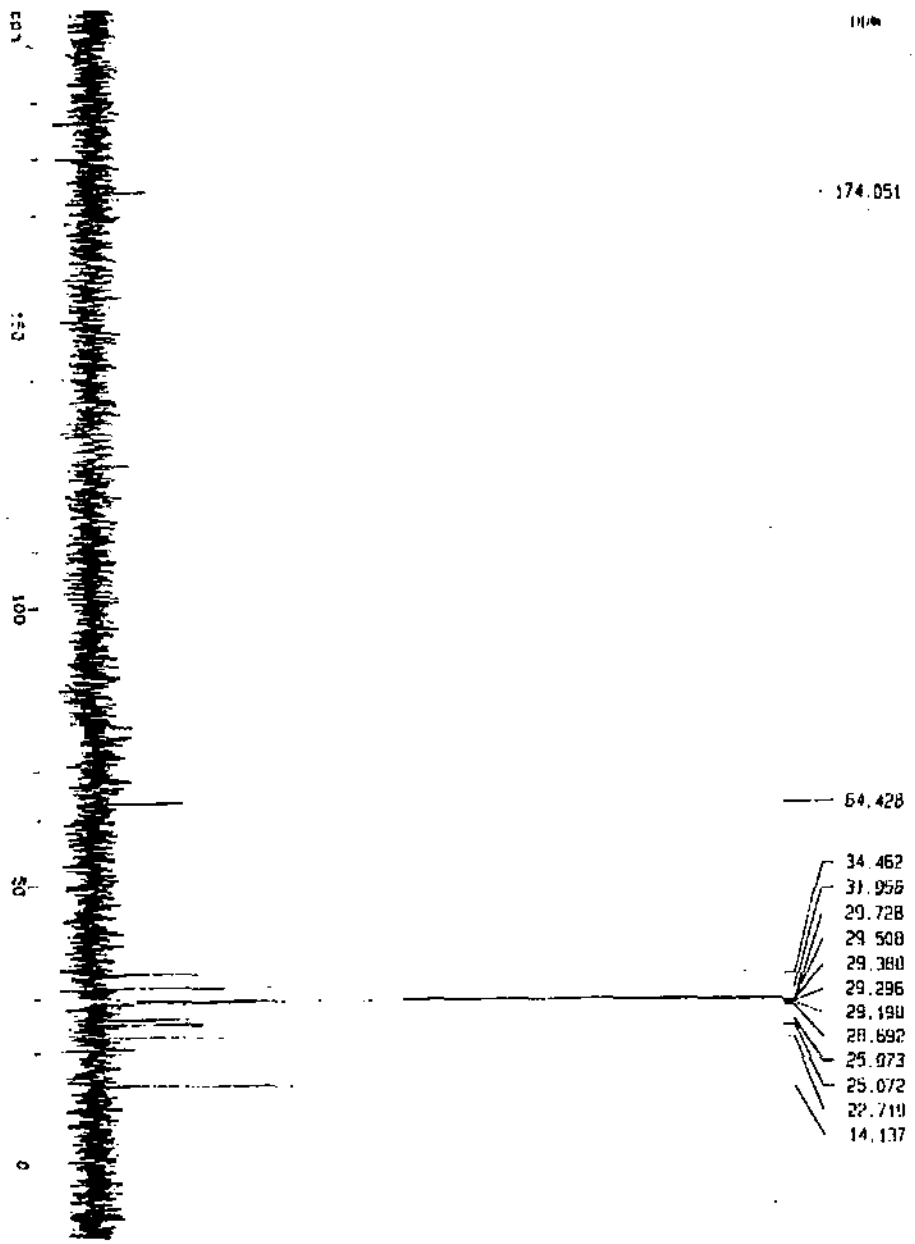


Figure- 3.23: ^{13}C-NMR spectrum of compound V_2

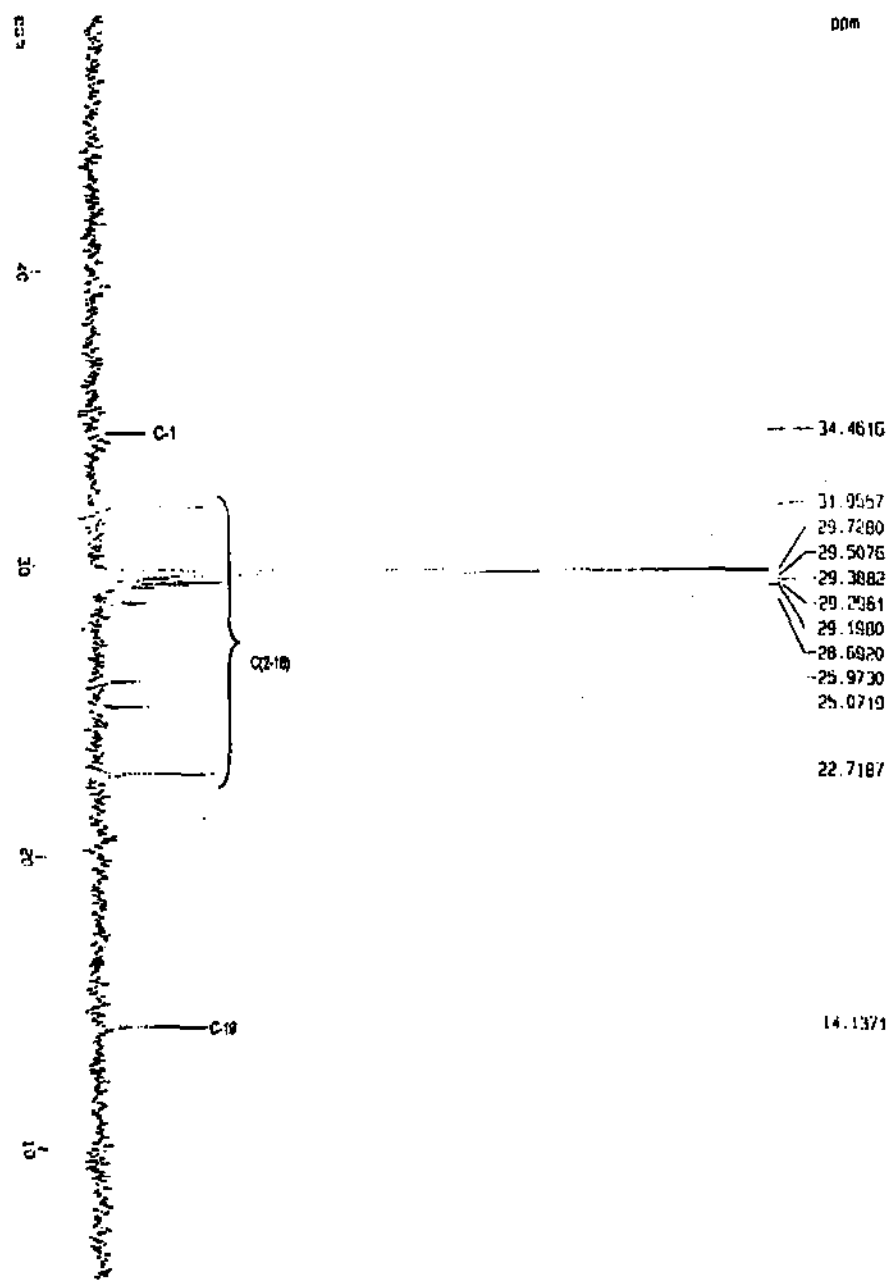


Figure- 3.24: Expended ^{13}C-NMR spectrum of compound V_2

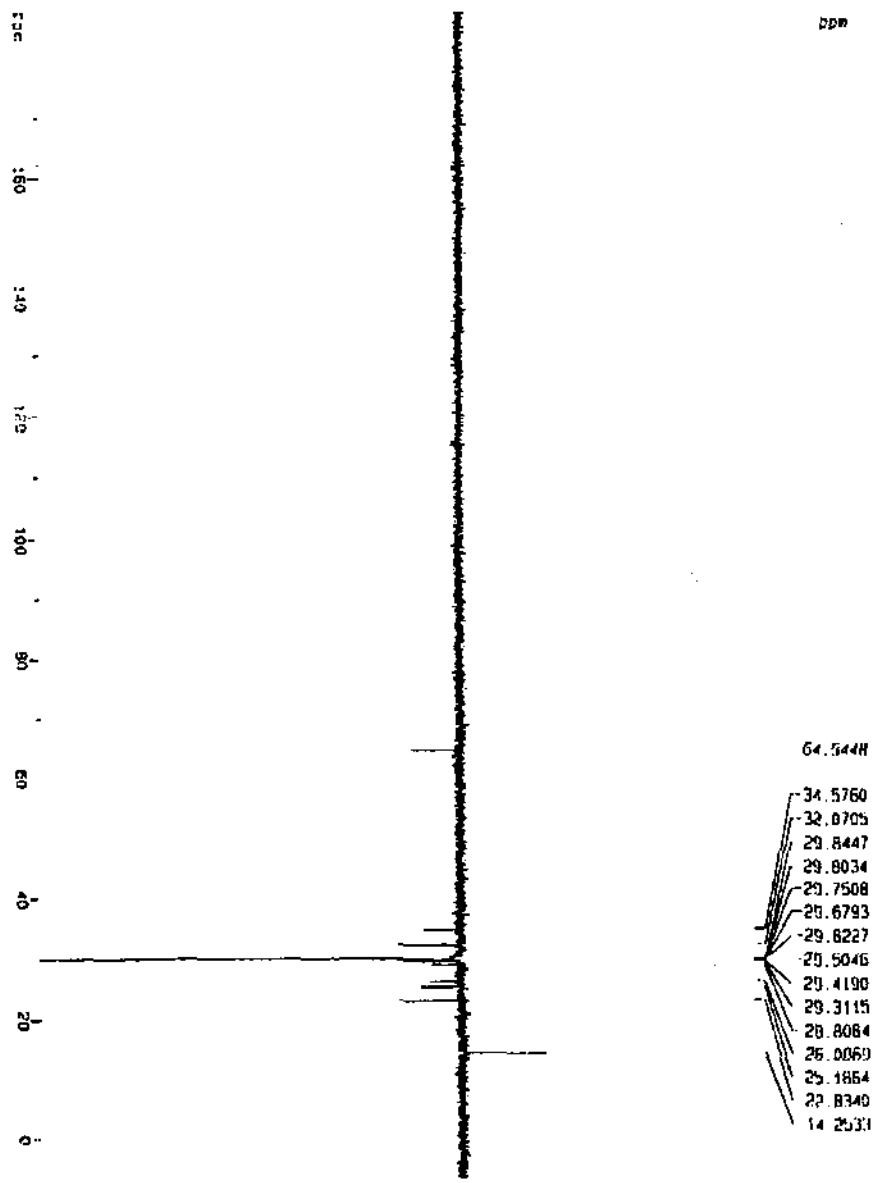


Figure- 3.25: Dept 135 spectrum of compound V_2

The E1 mass spectra (fig: 3.26 and Data 3.27) of the compound V_2 showed m/z 285 (M+1) indicating its molecular formula $C_{19}H_{40}O$. The melting point of V_2 was compared with reported melting point of 1-Nonadecanol[42] and it was same.

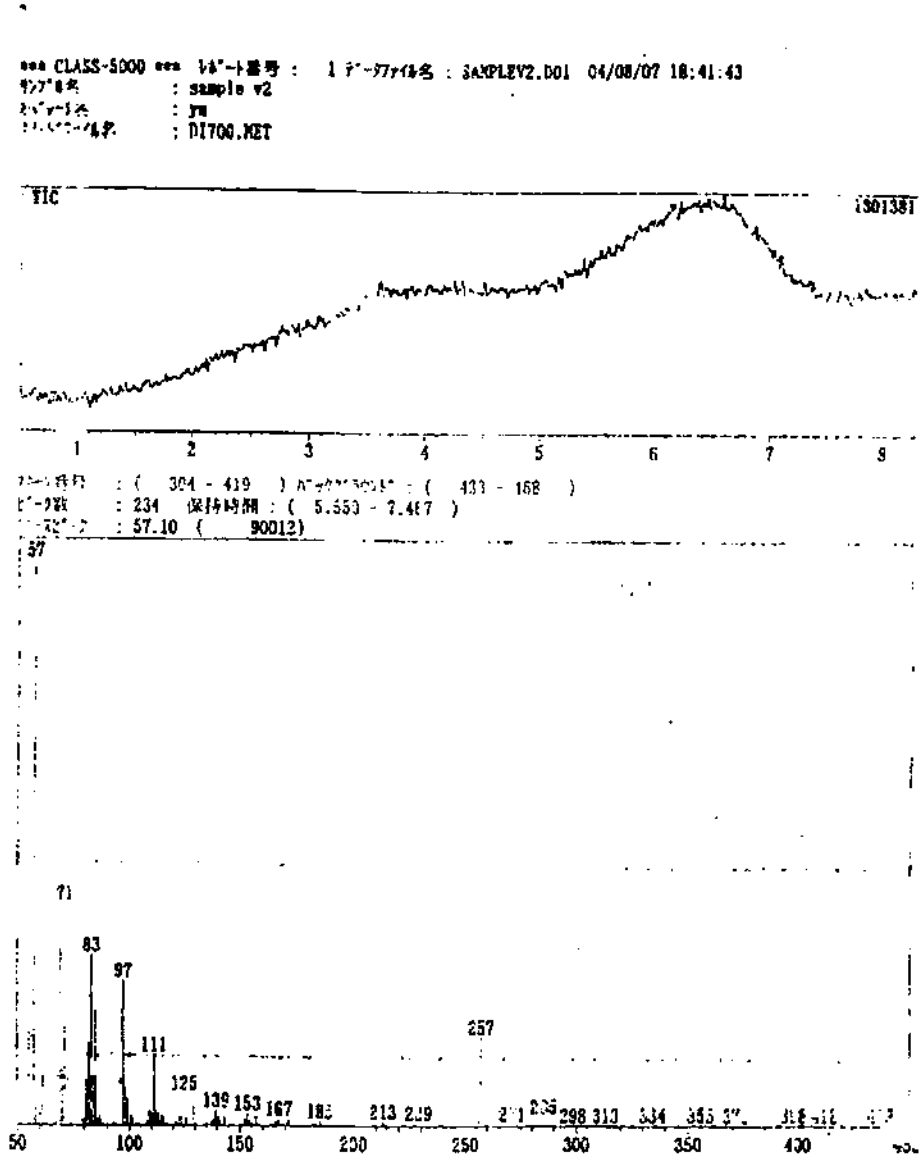


Figure- 3.26: Mass Spectrum of V_2

データファイル名 : SAMPLEV2.D01
スキャン番号 : (304 - 419) バックグラウンド : (433 - 468)
ピーク数 : 74 保持時間 : (5.550 - 7.467)

質量数	相対強度	質量数	相対強度
54.10	5.59	112.05	2.66
55.10	42.39	113.10	3.32
56.10	18.37	114.05	0.74
57.10	100.00	115.05	1.73
58.10	5.65	116.05	1.25
59.10	1.39	121.10	0.73
60.05	5.79	123.05	1.89
61.10	8.76	124.05	1.55
64.00	0.63	125.20	5.51
67.10	7.34	126.10	1.87
68.10	8.34	127.10	1.41
69.10	30.11	129.10	4.28
70.10	10.48	130.10	0.55
71.15	38.00	137.10	1.17
72.10	2.39	138.10	1.15
73.10	7.86	139.10	2.58
79.10	1.26	140.10	1.36
80.10	1.02	141.10	0.88
81.10	7.80	143.10	1.38
82.10	13.76	152.10	1.87
83.10	29.12	153.10	2.08
84.10	8.31	154.10	0.91
85.15	19.33	157.10	1.52
86.10	1.20	166.10	0.77
87.10	2.04	167.10	1.13
88.05	0.51	169.10	0.51
89.05	1.08	171.10	0.89
95.15	5.17	181.10	0.59
96.10	7.83	185.10	0.77
97.15	24.52	195.05	0.64
98.15	6.41	213.00	0.75
99.15	4.60	228.95	0.51
101.05	1.74	237.00	0.56
108.05	0.91	256.00	2.96
109.20	2.61	257.35	15.00
110.15	2.14	258.35	3.18
111.20	11.96	265.30	1.67

Figure- 3.27: Data of Mass Spectrum of Compound V_2

The IR, ^{1}H-NMR, ^{13}C-NMR mass spectral analysis and melting point established the identity of compound V_2 as 1-Nonadecanol.

CH_3-[-CH_2-]$_{17}$-CH_2-OH

Structure of Compound V_2

3.6 CHARACTERIZATION OF COMPOUNDS ISOLATED FROM ETHYL ACITATE EXTRACT OF LEAVES OF *AMARANTHUS SPINOSUS (L.)*

3.6.1. CHARACTERISATION OF ISOLATED COMPOUNDS.

The compound H_1 was negligible in amount and here its characterization was not further carried out.

Characterization of compound H_2.

The compound H_2 was isolated as off-white crystalline powder having melting point 62-64^{0}C. [Section-2.5.4.4.1.4] The TLC examination of this isolated compound showed a brown coloured single elongated spot (R_f value =50: EA: MeOH=9: 1; silica gel) iodine chamber. The elongation of the spot indicates the compound had a polar part and a non-polar part.

The spot turned into violet on spraying with vanillin sulphuric acid followed by heating in an oven at 110^{0}C for about 10 minutes.

IR Spectrum Analysis

The analysis of IR spectrum[34,35] of the compound H_2 (Fig-3.28) exhibited a broad absorption bond at 3420 cm^{-1} indicative the presence of OH stretching of carboxylic acid. The two absorption peaks at 2930 cm^{-1} and 2850 cm^{-1} were due to the presence of aliphatic C-H stretching of either both -CH_3, -CH_2 or - CH group. The peak at 1690 cm^{-1} for C=O group of carboxylic acid.

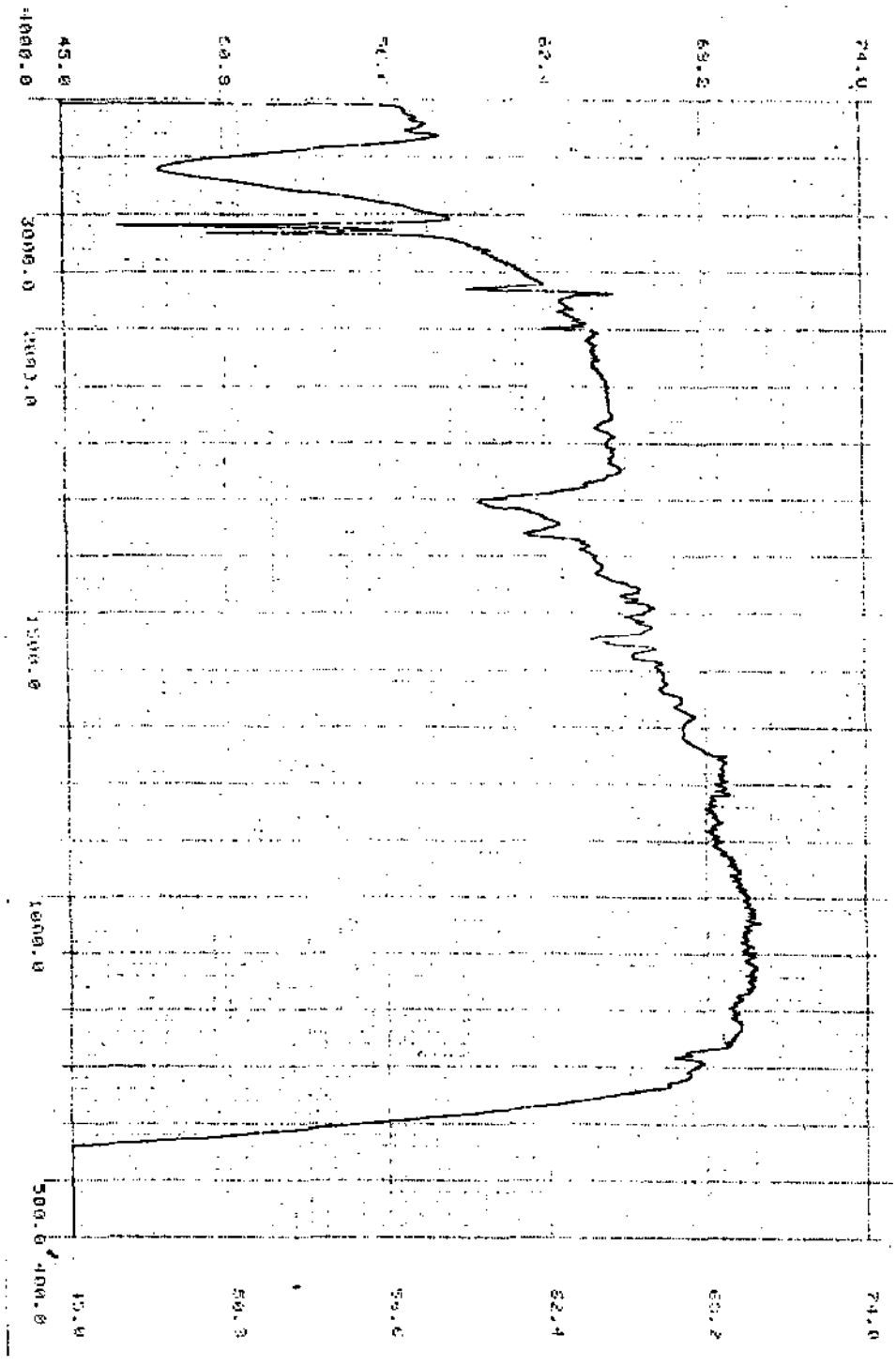


Figure- 3.28: IR Spectrum of Compound H_2

The two absorption bands at 1450 cm^{-1} and 1370 cm^{-1} were demonstrative of C-H bending of methylene (-CH_2-) and methyl (-CH_3) group respectively.

^{1}H-NMR Spectra Analysis

The ^{1}H-NMR spectral[34,35] data (Fig-3.29 & 3.30) of compound H_2. showed that the protons at δ 0.86-0.87 ppm as triplet, which indicates the three protons of methyl group at C-22. The multiplet at δ 1.25 ppm indicative 36 protons at C-4 to C-21. The multiplet at δ 1.624 ppm indicative two methylene protons at C-3. The triplet at δ 2.32-2.35 ppm was due to two methylene protons at C-2.

Table 3.5: ^{1}H-NMR spectral[34,35] data assignment of compound H_2 (400 MHz; in $CDCl_3$; TMS as standard)

Chemical shift δ-value in ppm	Splitting pattern	Assignment
0.86 - 0.87	triplet	three methyl protons at C-22
1.25	multiplet	36 equivalent protons at C-4-C-21
1.624	multiplet	two methylene protons at C-3
2.32-2.35	triplet	two methylene protons at C-2

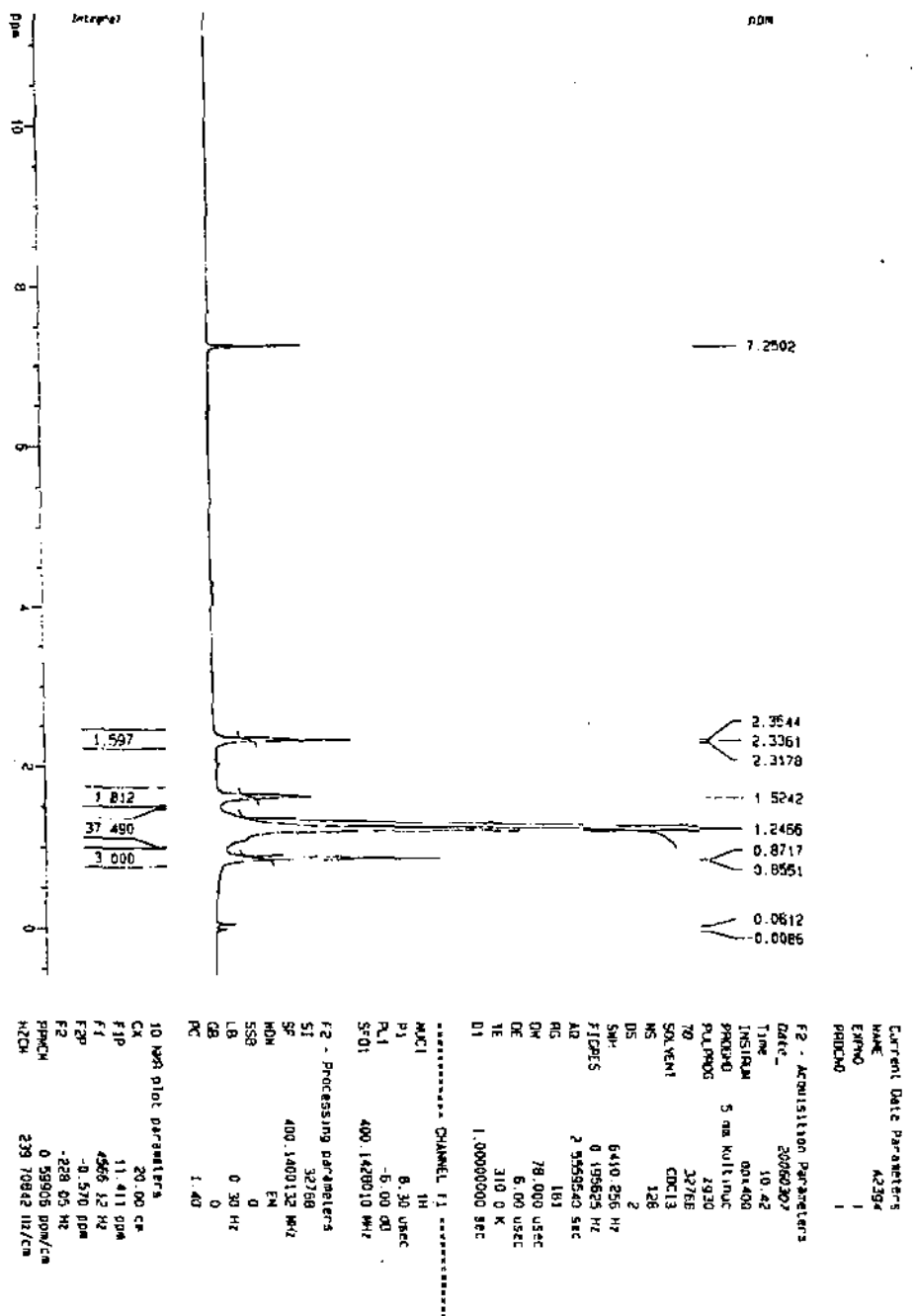


Figure- 3.29: ^{1}H-NMR Spectrum of Compound H_2

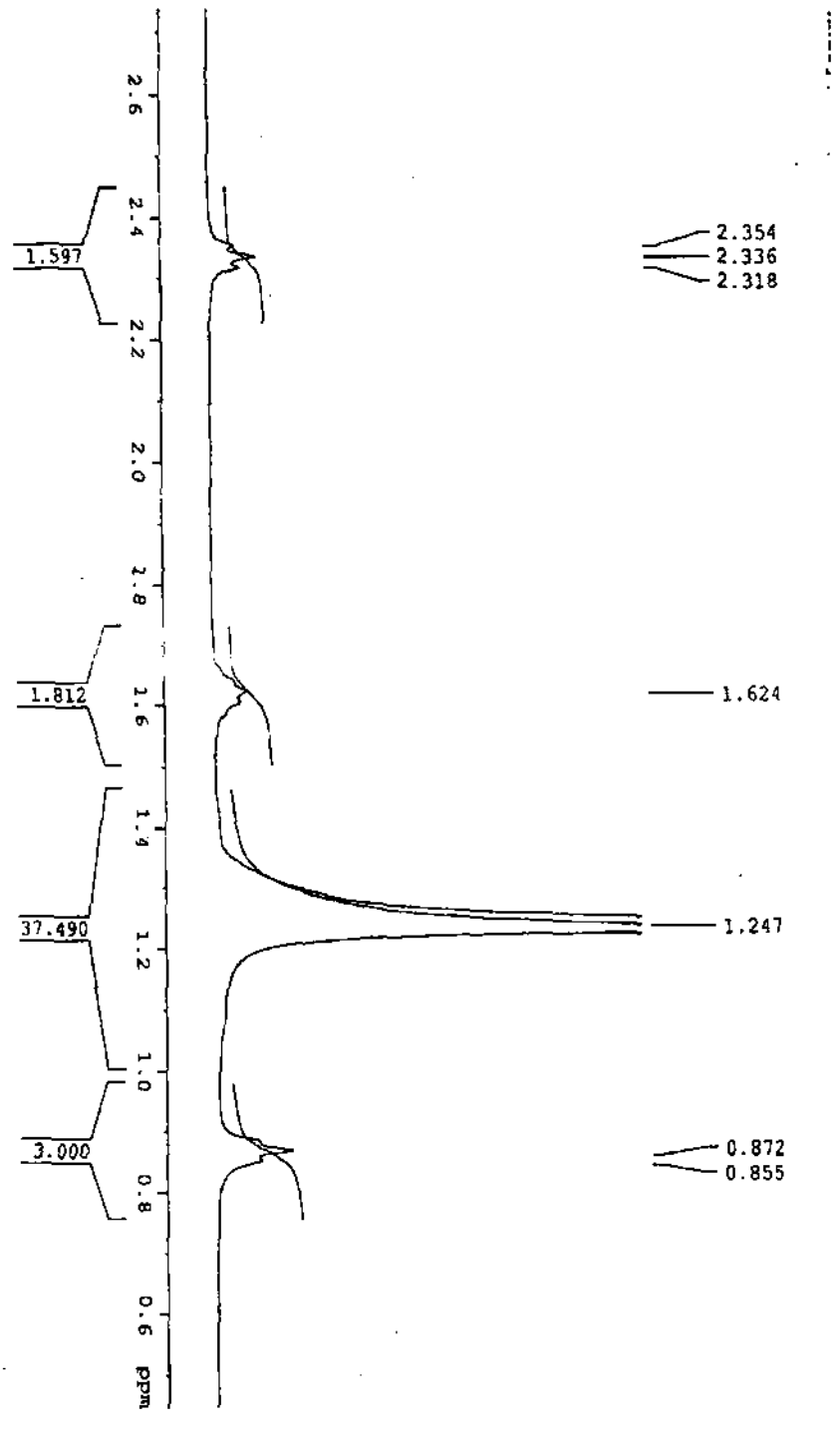


Figure- 3.30: Expended ^{1}H-NMR Spectrum of Compound H_2

The IR and ^{1}H-NMR spectral data indicated that the compound H_2 may be a saturated fatty acid. The ^{1}H-NMR spectral data of the compound H_2 was compared with ^{1}H-NMR data of a fatty acids[41] and the tentative structure of the compound H_2 is proposed as below-

0.86-0.87	1.25	1.624	2.32-2.35

CH_3-$(CH_2)_{18}$-CH_2-CH_2-COOH

22	4-21	3	2	1

The compound H_2

According to the proposed tentative structure the name of the compound H_2 is docosanoic acid.

3.6.2. CHARACTERISATION OF ISOLATED COMPOUND M_1, M_2 and M_3

The compounds M1 and M2 obtained were very negligible in amount so their further characterization have not done.

The compound M_3 was isolated from the fraction F_4 obtained upon VLC separation of ethyl acetate extract of the leaves of *Amaranthus spinosus* followed by preparative TLC.

Its TLC showed an elongated single spot (R_f value=0.62; petroleum ether: dichloromethane=4:1 ; silica gel). Upon exposure to iodine vapour a brown coloured spot was appeared. The spot turned into violet colour on spraying with vanillin-sulphuric acid followed by heating in an oven at 110^{0}C for 10 minutes. The elongation of the spot is thought to be indicative of a compound with a polar part undergoing strong interaction with silica gel and remains at the lower part of the elongated spot while the non-polar part is moved by solvent towards the upper part of the spot resulting in its elongation (tailing).

IR spectrum analysis

The IR spectrum of the compound M_3 (Fig-3.31) exhibited a broad absorption band at 3450 cm^{-1} indicating the presence of a hydroxyl, (-OH) group.

The tiny absorption peak at 3050 cm^{-1} was for H-C=C-skeleton[35]. The two absorption peaks at 2930 cm^{-1} and 2850 cm^{-1} were due to the presence of aliphatic C-H stretching of either-CH_3, - CH_2 or -CH group. The two peaks at 1700 cm^{-1} and 1725 cm^{-1} were due to the ester carbonyl group[36].

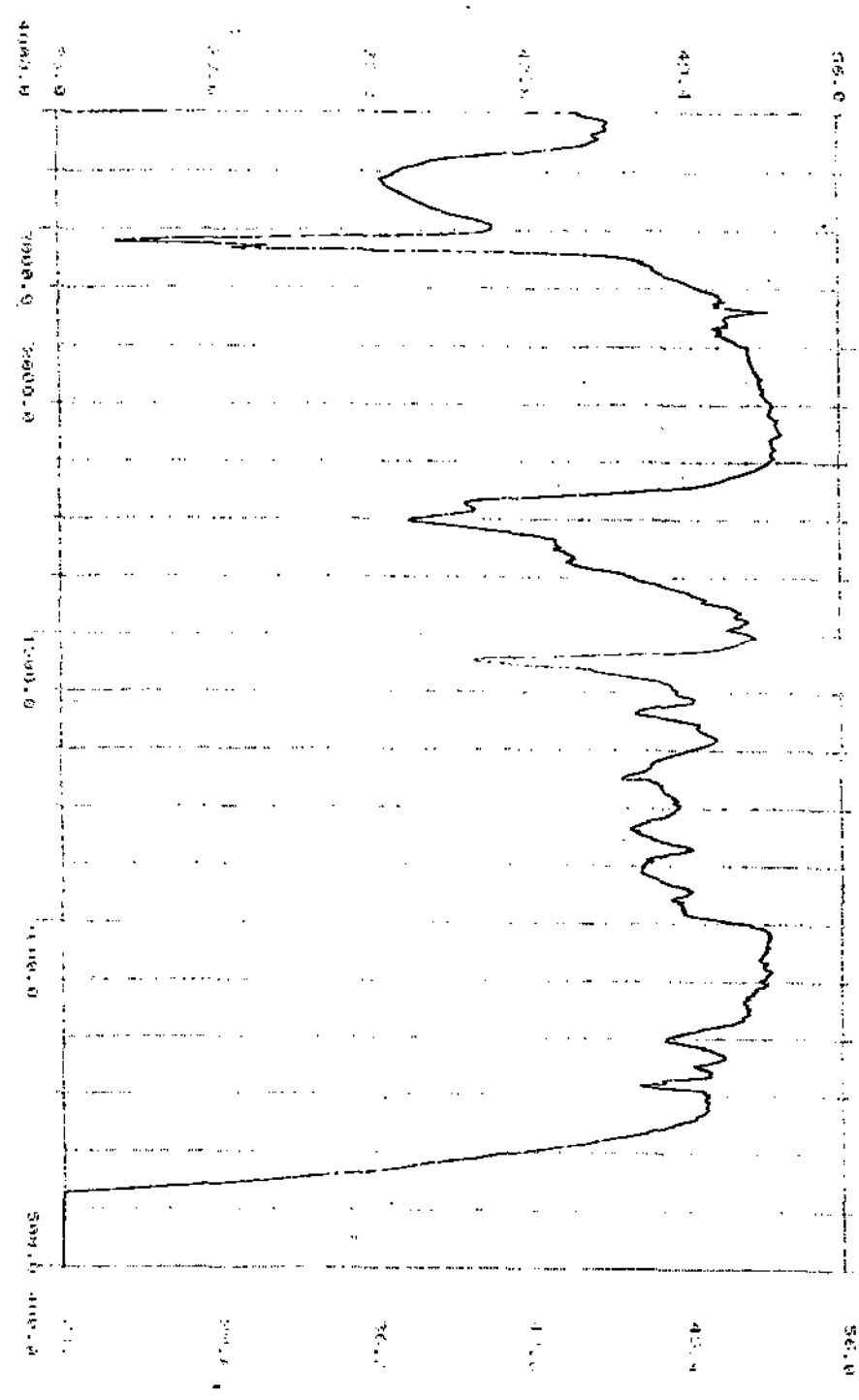


Figure- 3.31: IR Spectrum of Compound M_3

The peak at 1640 cm^{-1} was due to a >C=C< double bond skeletal unit. The two absorption bands at 1460 cm^{-1} and 1375 cm^{-1} were due to C-H bending of methylene ($-CH_2-$) and methyl ($-CH_3$) groups respectively. The absorption band at 1250 cm^{-1} to 1050 cm^{-1} was due to the presence of C-O stretching for alcoholic group. The peak at 810 cm^{-1} was also due to the presence of a >C=C< double bond.

^{1}H-NMR spectra analysis

The ^{1}H-NMR (400 MHz, in $CDCl_3$; TMS as standard) spectrum (Fig-3.32, 3.33 and 3.34) of the compound M_3 had a sharp triplet of 1-H intensity at δ 1.99 ppm indicative of the presence of methine proton at C-1. The triplet at δ 1.58 ppm and δ 2.27 ppm were indicative of the presence of two non-equivalent protons of methylene group at C-6.

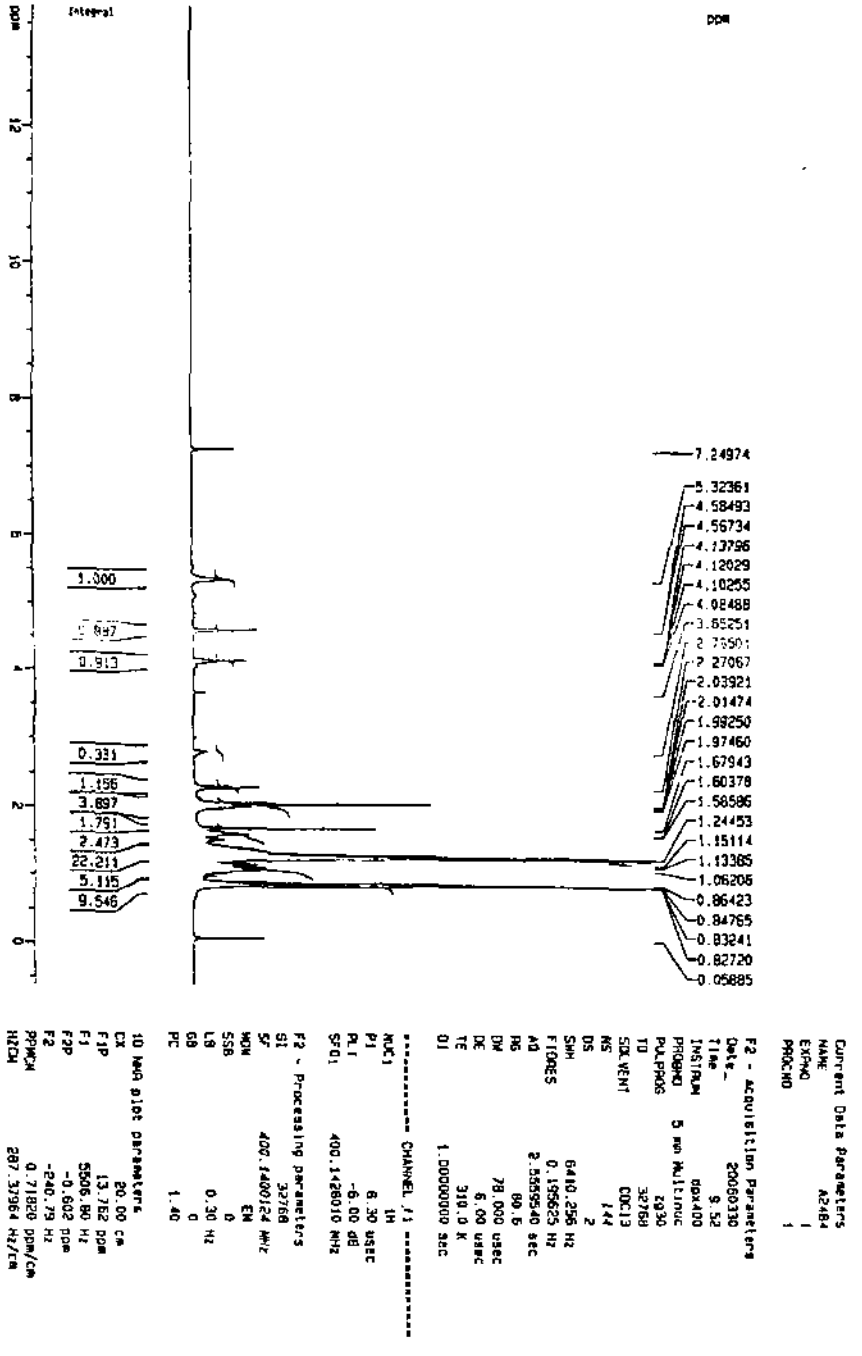


Figure- 3.32: ^{1}H-NMR Spectrum of Compound M_3

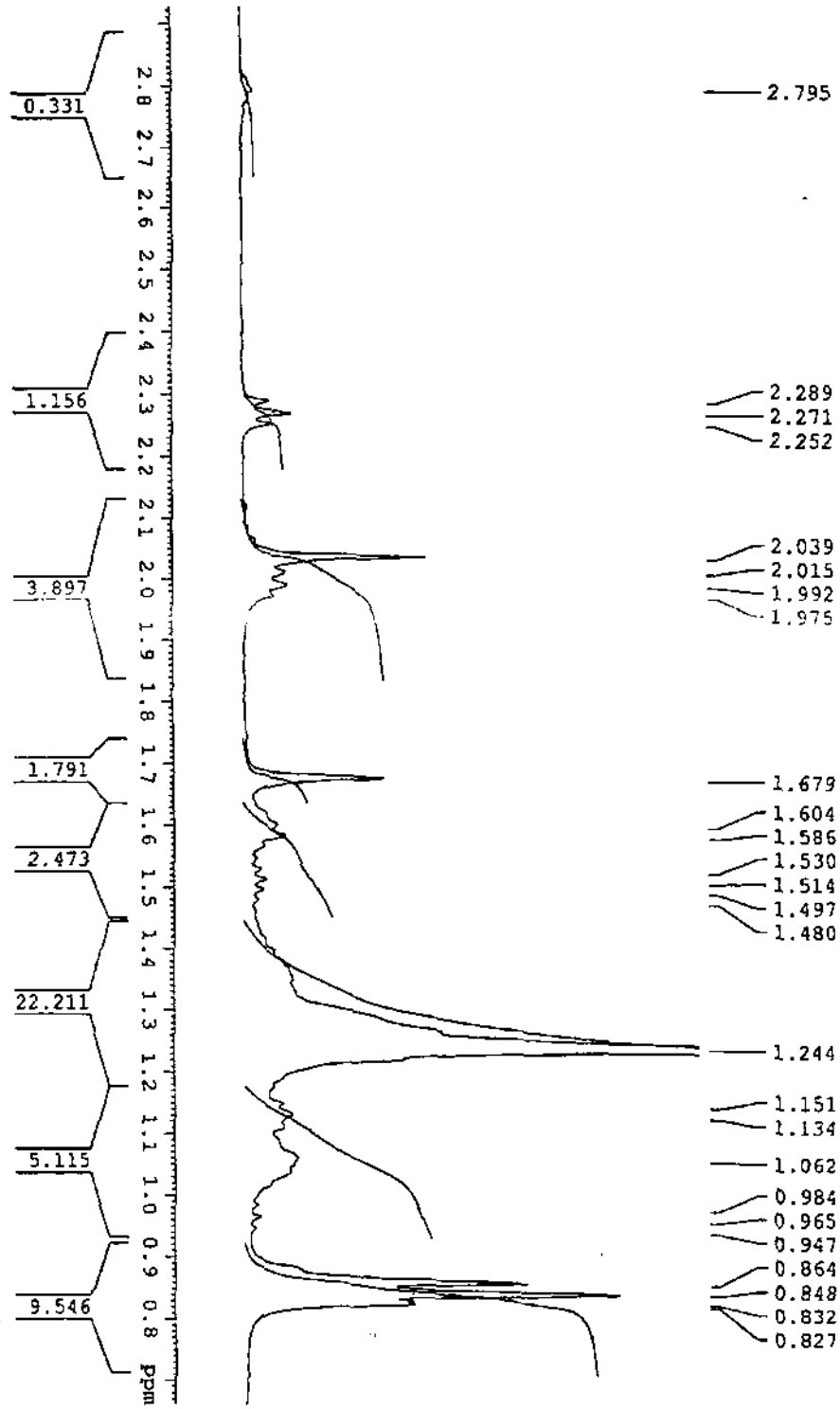


Figure- 3.33: Expended ^{1}H-NMR Spectrum of Compound M_3

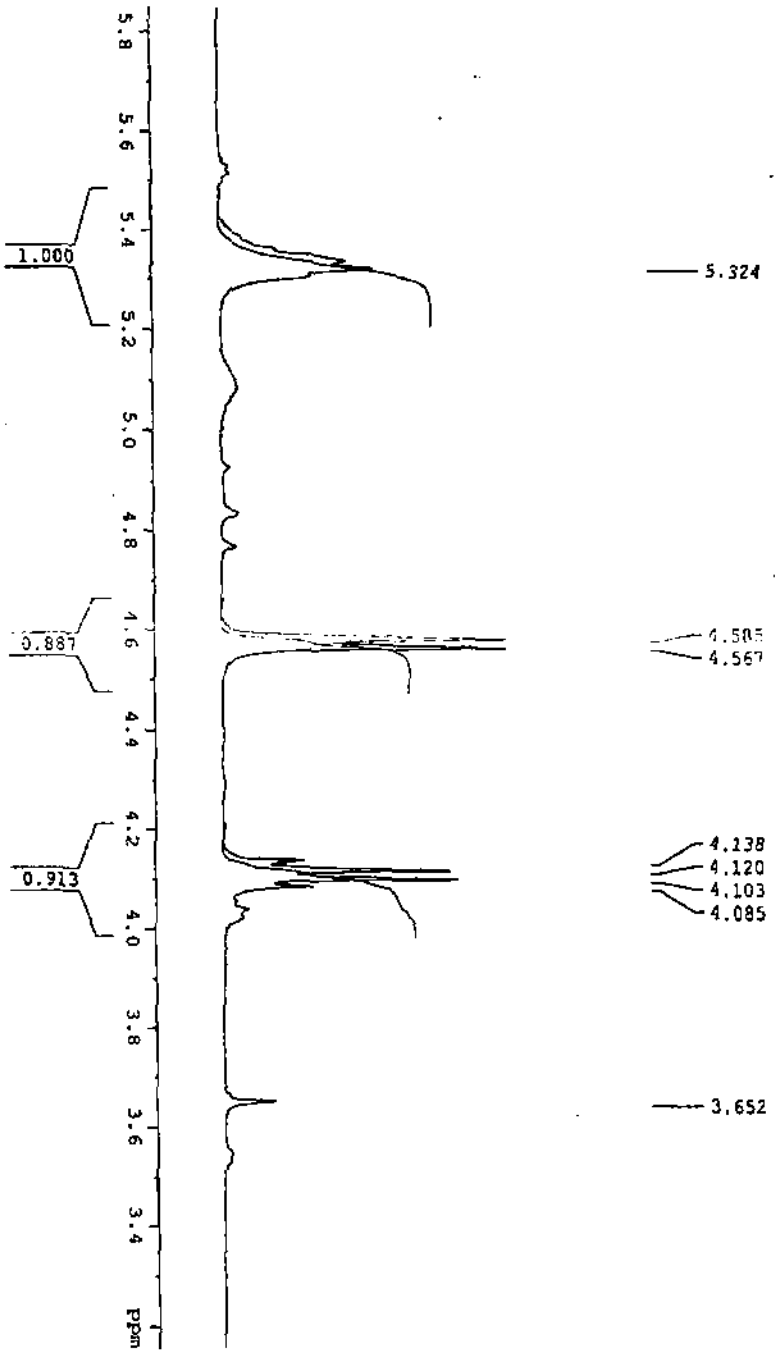


Figure- 3.34: Expended ^{1}H-NMR Spectrum of Compound M_3

The broad singlet at δ 1.67 ppm was assignable as methylene protons at C-5. The singlet at δ 2.01 ppm was indicative three methyl protons at C-10. The singlet at δ 3.63 ppm was due to hydroxyl proton at C-2. The sharp triplet45 at δ 0.82 ppm was indicative the presence of three protons at C-9. The quartet at δ 4.1 ppm was indicative of the presence of two methylene protons at C-8. The doublet at δ 4.5 ppm was due to an oxymethine proton at C-2. The multiplet at δ 5.3 ppm was of indicative of the presence of one olefmic proton at C-4.

Table 3.6: ^{1}H-NMR spectral data[34,35] assignment of the compound M_3 (400 MHz; in $CDCl_3$; TMS as standard).

Chemical shift δ-value in ppm	Splitting pattern	Assignment
0.82	triplet	three methyl protons at C-9
1.58	triplet	One non-equivalent proton at C-6
1.67	broad singlet	two equivalent methylenic protons at C-5
1.99	Triplet	One methine proton at C-5
2.1	singlet	three methyl protons at C-10
2.27	triplet	one non-equivalent proton at C-6
3.63	singlet	Alcoholic proton at C-2
4.1	quartet	two methylene protons at C-8
4.5	doublet	one methine proton at C-2
5.3	multiplet	one olefinic proton at C-4

^{13}C-NMR spectroscopy M_3:

The ^{13}C-NMR spectrum (Fig-3.36) of isolated compound, M_3 showed the presence of twenty six (26) carbon signal. The signals at δ 173.96 ppm was indicative of the presence of ester carbon at C-7. The signals at δ_C 142.78 and 118.0 ppm were indicative of the presence of two olefinic carbons C-3 and C-4. The tiny signal at δ_C 69.2 ppm was indicative of the presence of hydroxy methine carbon C-2. The signal at 61.46 ppm was indicative of the presence of C-8 carbon attached to oxygen of the carboxylate moiety. The signal at δ_C 32.80 ppm was indicative of the presence of methine carbon at C-1. The signal at δ_C 39.87 and 37.44 ppm due to methylene carbon at C-6 and C-5. The signal at δ_C 21.06 and 19.75 ppm were indicative of the presence of methyl carbon at C-10 and C-9.

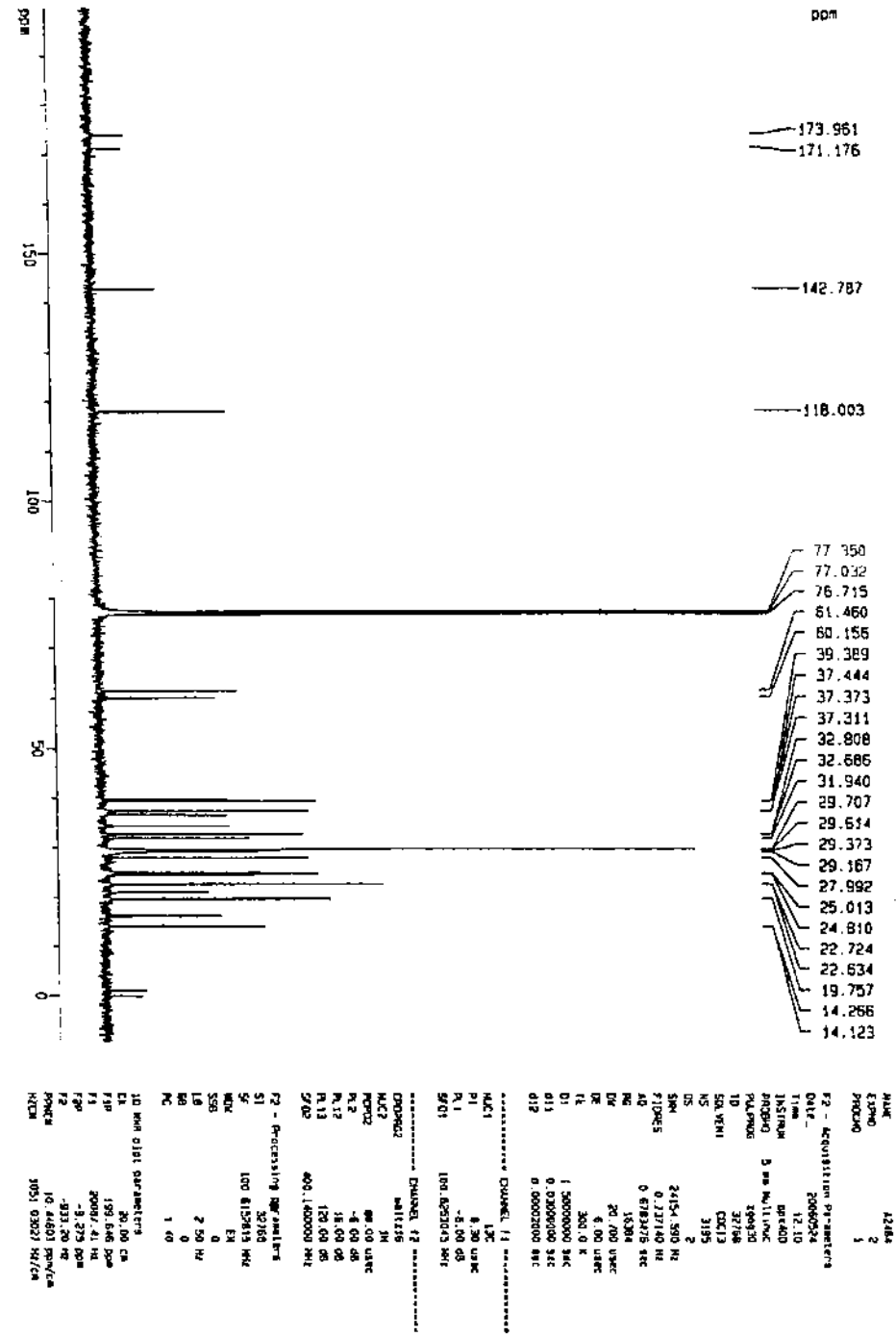


Figure- 3.35: ^{13}C-NMR Spectrum of Compound M_3

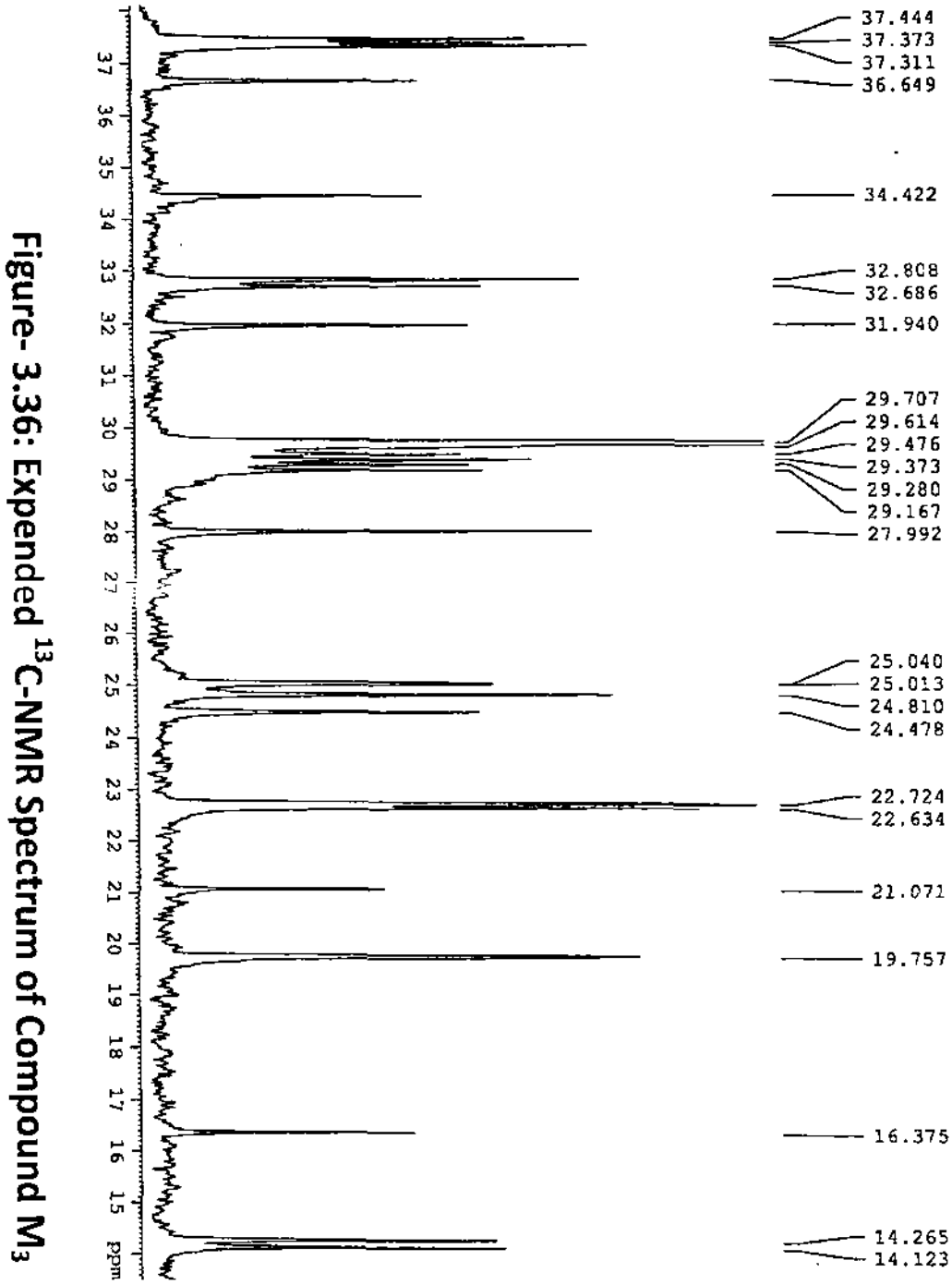


Figure- 3.36: Expended ^{13}C-NMR Spectrum of Compound M_3

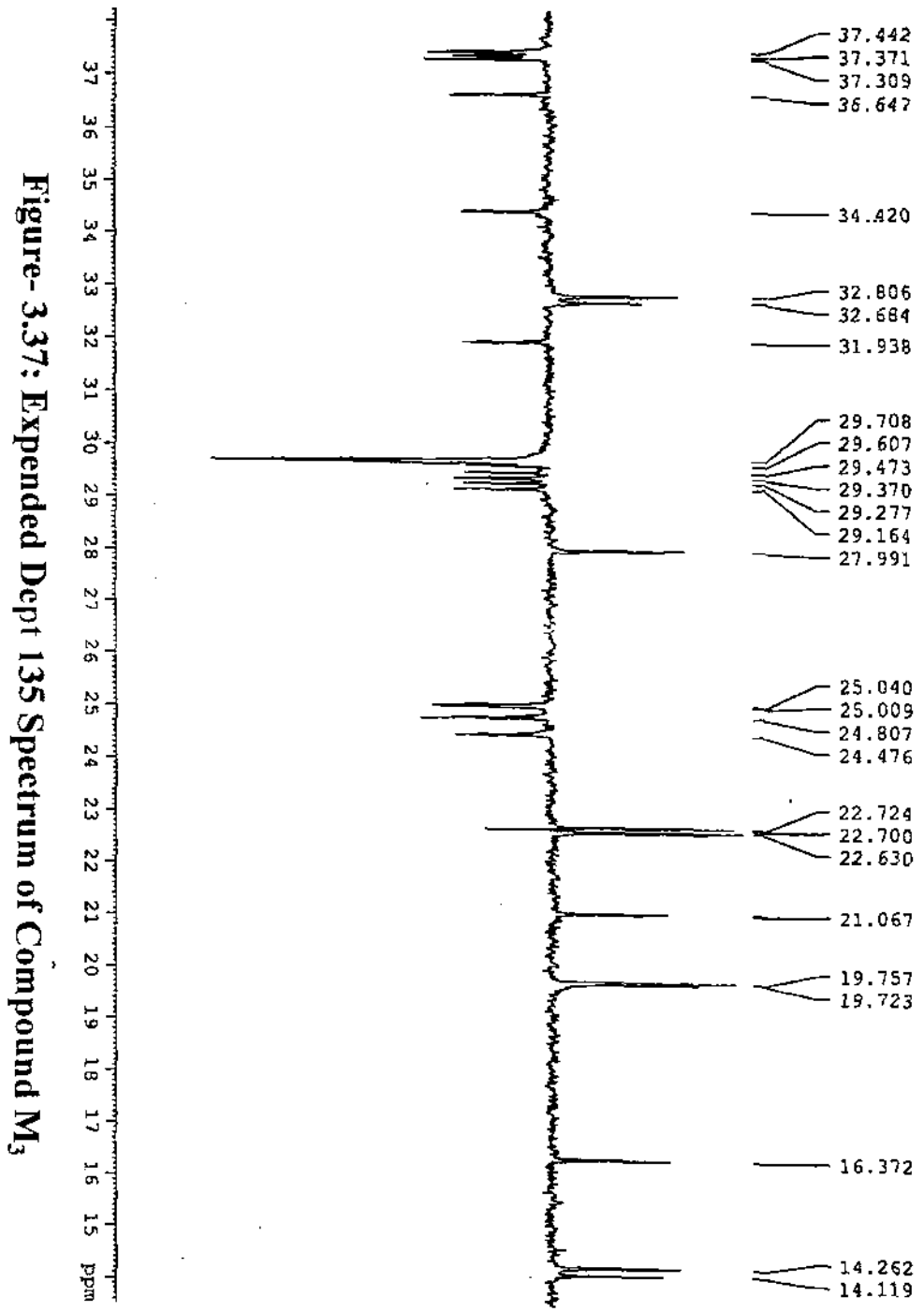
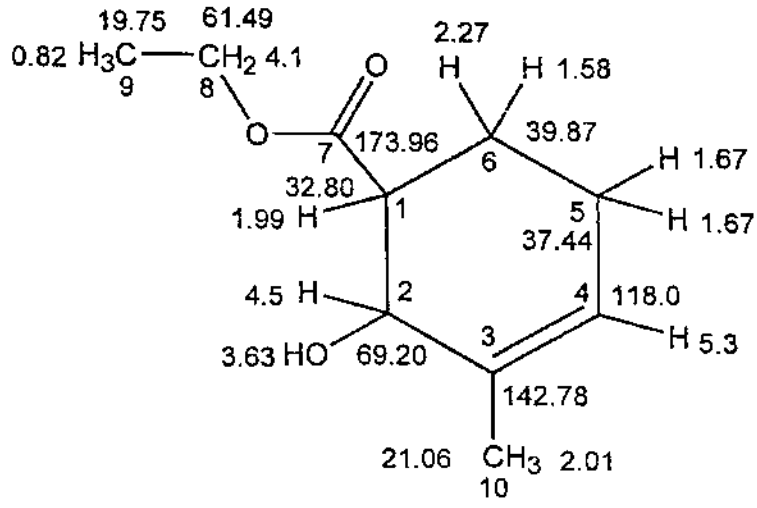


Figure- 3.37: Expended Dept 135 Spectrum of Compound M_3

Table-3.7: ^{13}C-NMR Spectral data of the isolated compound M_3.

Carbon No.	Types of carbon	Chemical shift in ppm
1	>CH-	32.80
2	H-C-O-H	69.2
3	= C <	142.78
4	= C < H	118.0
5	$-CH_2-$	37.44
6	$-CH_2-$	39.87
7	R-COOR	173.96
8	$-CH_2-O-$	61.49
9	$-CH_3$	19.75
10	$-CH_3$	21.06

Based on IR, ^{1}H-NMR and ^{13}C-NMR spectral data the tentative structure of the compound 'M_3' was proposed as-



The compound M_3

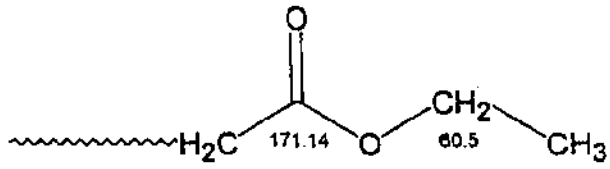
The compound M_3 has been named as Ethyl 2-hydroxy -3- methyl cyclohex-3-ene carboxylate.

However, from IR, ^{1}H-NMR and ^{13}C-NMR spectral data, it has been found that the compound was not completely pure. Another minor compound was mixed with the major compound.

The ^{1}H-NMR spectrum showed that the triplet at δ 0.94-0.96 ppm was of indicative of the 3H of methyl group. Again the multiplet at δ 1.246ppm was of indicative of 'n' number of methylene (-CH_2) group.

The ^{13}C-NMR spectrum showed that the isolated compound has twenty six (26) carbon signals. Among them ten (10) carbon signals were accommodated for major compound. Thus the rest of the signals were for the minor compound. The signals at δ_C 171.17 and 60.15 ppm were indicative of the presence of carbonyl carbon of ester and carboxy methylene (-O-CH_2) carbon respectively.

Therefore, the minor one may have the following structural units.



As the compound contains minor amount of impurities so, its complete structure determination needs it purification but it was not possible within this time frame

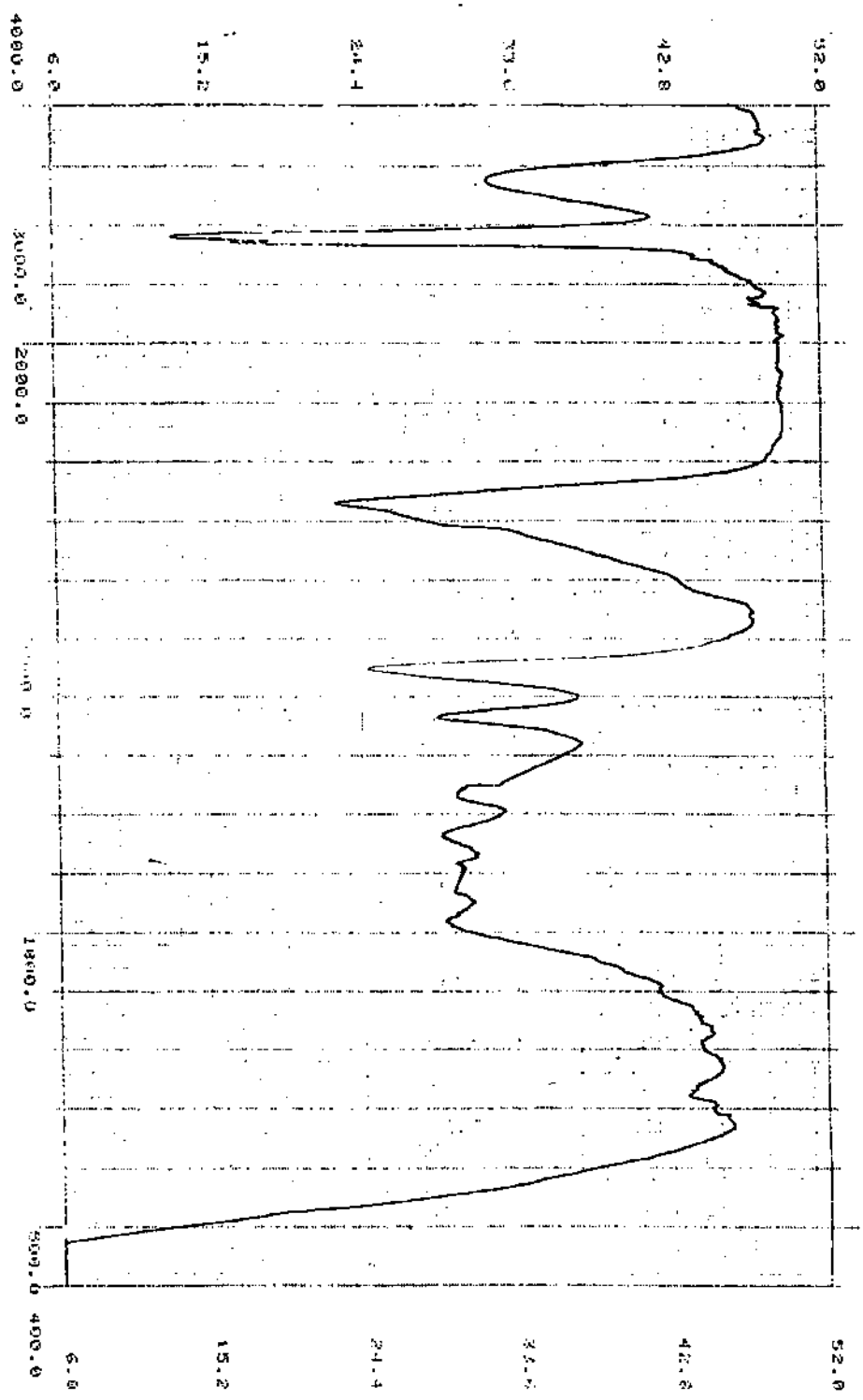


Figure- 3.38: IR Spectrum of Compound M_1

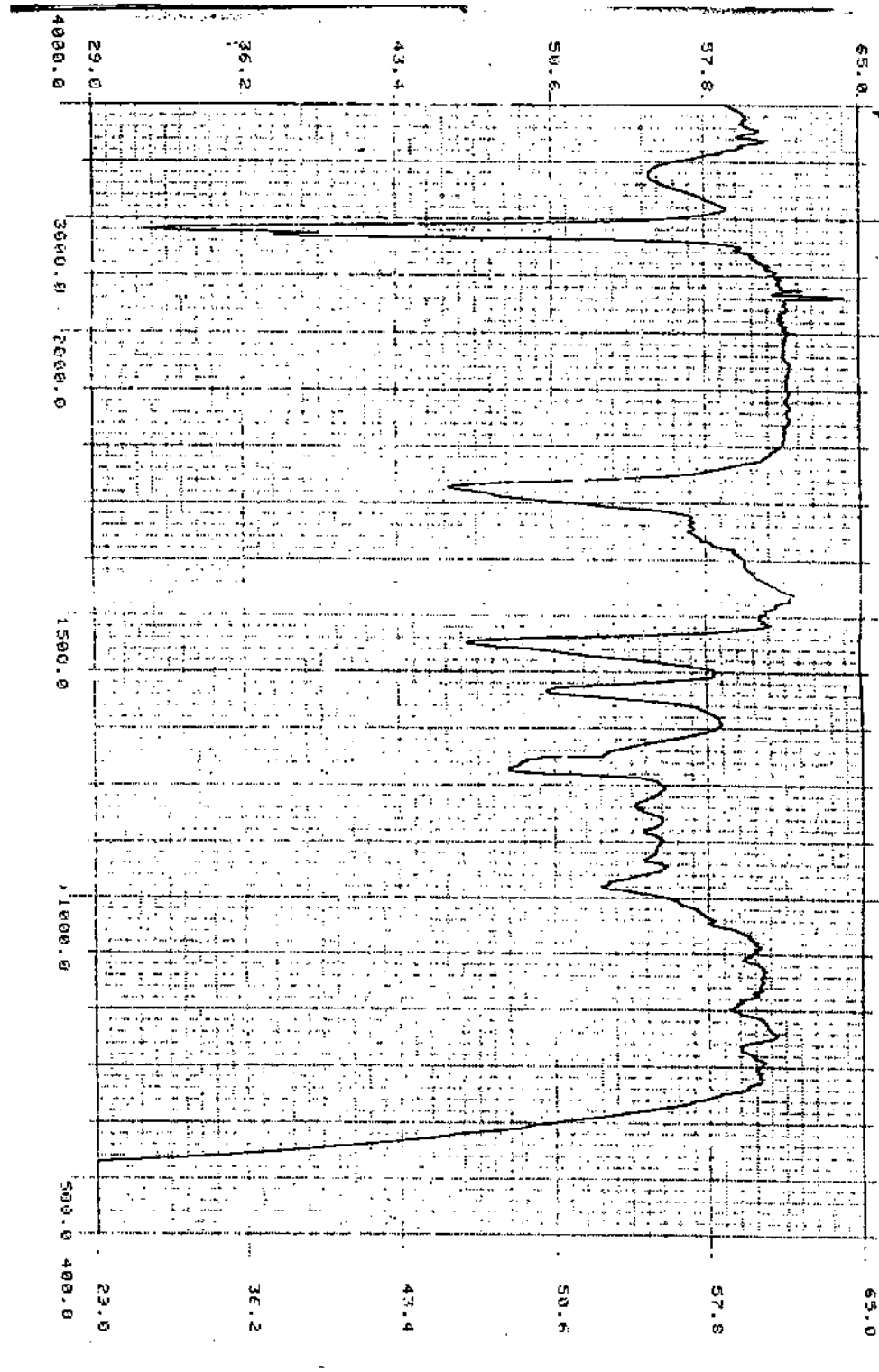


Figure- 3.39: IR Spectrum of Compound M_2

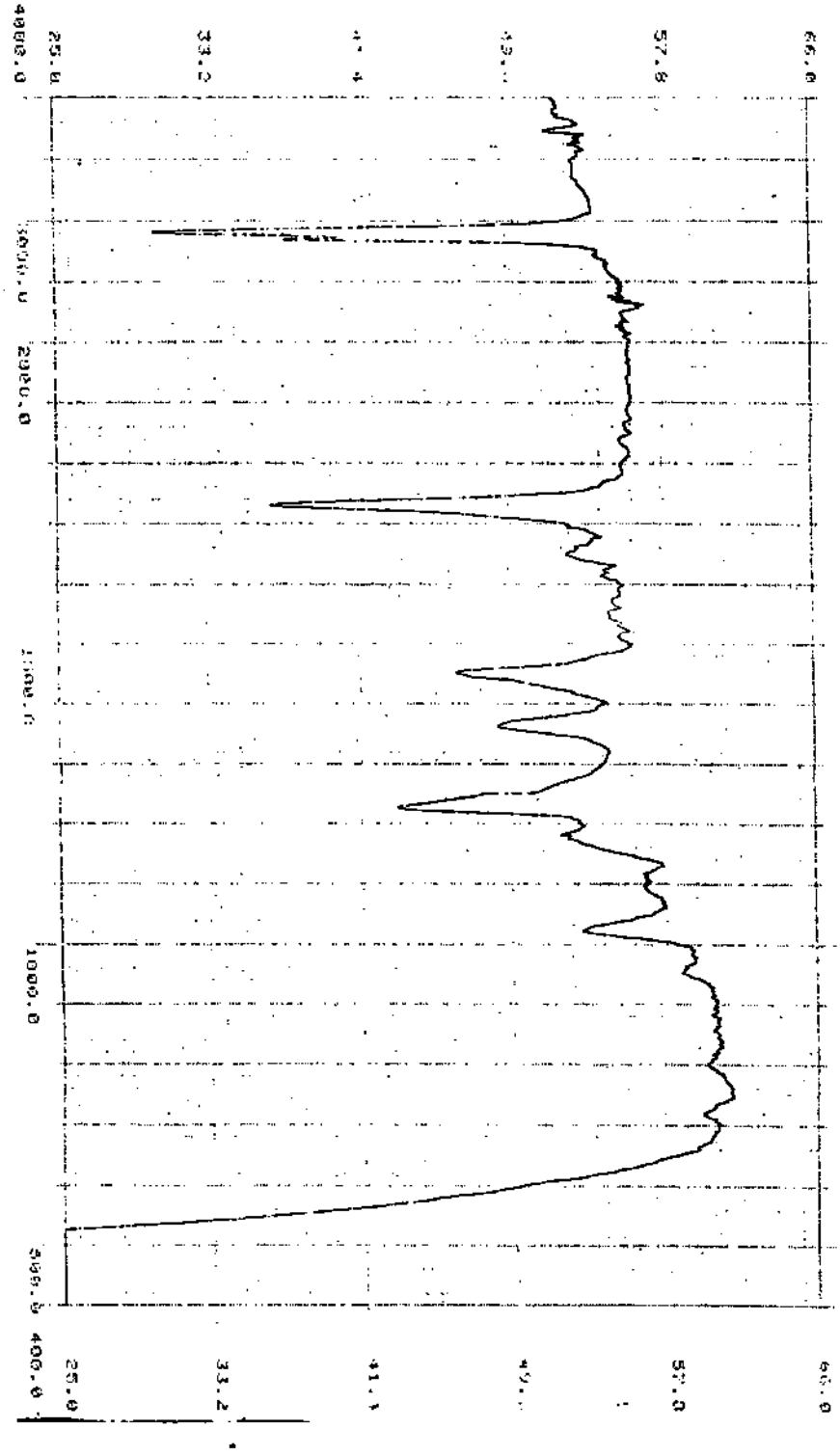


Figure- 3.40: IR Spectrum of Compound H_1

CHAPTER FOUR

ASH CONTENT ANALYSIS

4. ASH CONTENT ANALYSIS

4.1 ASH

The ASH content is the percentage of inorganic residue remaining after ignition of sample.

4.2 PROCEDURE:

A weight quantity of sample was taken in a crucible. The contents were heated carefully to the ignition point and allowed to burn spontaneously. When the burning was completed, the crucible heated at a dull-red heat in a Muffle-furnace at 600^0C for three hours. The crucible was then cooled and weighed with an accuracy of 1 mg[48].

4.3 CALCULATION:

$$\text{ASH, percentage} = \frac{100 \times W2}{W1}$$

Where, W_1 = Initial wt. of the sample in gm =2 gm.

W_2 = wt. of the ASH in gm.

4.4 RESULTS:

The results of the determination of ASH content of the leaves of ***Amaranthus Spinosus*** are given in the table-4.1

Table-4.1

The ash content of the leaves of *Amaranthus spinosus*.

Crucible no	Weight of crucible (gm)	Weight of crucible+ash (gm)	Wt. of ash gbtained (gm)	Percentage (%)	Average Percentage (%)
1	21.05	21.85	0.80	40	
2	21.75	22.45	0.70	35.44.17	
3	19.75	20.90	1.15	57.5	

CHAPTER FIVE

IRON CONTENT ANALYSIS

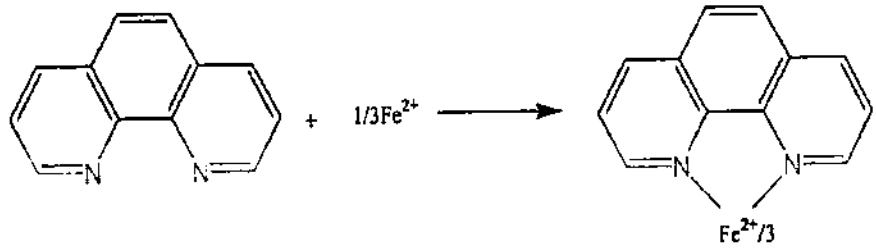
5. IRON CONTENT ANALYSIS

5.1 PRINCIPLE:

A complex of iron (II) is formed with 1, 10- phenanthroline, Fe $(C_{12}H_8N_2)_3^{2+}$, and the absorbance of this colored solution is measured with a spectrophotometer[47]. The spectrum is plotted to determine the absorption maximum. Hydroxylamine (as the hydrochloride salt to increase solubility) is added to reduce any Fe_3+ to Fe^{2+} and to maintain it in that state.

Equations:

$$4Fe^{3+} + 2NH_2OH \longrightarrow 4Fe^{2+} + N_2O + 4H^+ + H_2O$$



1,10-phenantroline

tris(1,10-phenantroline) iron(II)

5.2 PROCEDURE:

Into a series of 100-ml volumetric flasks, 2.5, 5.0, 10.0, 20.0 ml of the standard iron solution was added47. Into another 100-ml volumetric flask 50 ml distilled water was placed for a blank. The unknown sample was furnished in another 100-ml volumetric flask. To each of the flasks (including the unknown) 1.0 ml of the hydroxyl ammonium chloride solution and 5.0 ml of the 1, 10- phenanthroline solution was added. Each solution was buffered by the addition of 8.0 ml of the sodium acetate solution to produce the red color of ferrous 1, 10-phenanthroline [The iron (II)- phenanthroline complex forms at pH 2 to 9]. The sodium acetate neutralizes the acid present and adjusts the pH to a value at which the complex forms. It was allowed for at least 15 minutes after adding the reagents before making absorbance measurements so that the colour of the complex can fully develop.

Each solution was diluted to exactly 100 ml. The standards were corresponding to 0.1, 0.2, 0.5 and 1 ppm iron, respectively. The blank solution was used as the reference solution. The absorbance against the wavelength was plotted and the wavelength of the absorption maximum was selected. A calibration curve by measuring the absorbance of each of the standard solutions at the wavelength (510nm) of maximum absorbance was measured. Similarly, the absorbance of the sample solution was measured. The absorbance of different standards was plotted against concentration in ppm. From this plot using the absorbance of the sample solution, the final concentration of iron (II) in the sample solution was determined and amount of iron in sample was reported.

5.3 RESULT:

Table 5.1: The absorbance of each of the standard solutions at the wavelength of the maximum absorbance (510nm).

Volume of standard Fe solution (ml)	Concentration of Fe solution (ppm)	Absorbance
2.50	0.1	0.225
5.00	0.2	0.279
10.00	0.5	0.381
20.00	1	0.581

The concentration of iron in the unknown samples were determined and given in the Table-6.2 .

Table-5.2: The concentrations of the unknown sample

Sample no	Absorbance at 510 nm	Conc. Of the unknowns (ppm)	Amount of Fe (mg) in 2 g dry mass	Percentage of the amount of Fe (%)	Average percentage of the amount of Fe (%)
I	0.205	0.5285	0.1322	6.61	
II	0.222	0.5725	0.1636	8.18	6.70
III	0.214	0.5518	0.1060	5.30	

Discussion: Iron, an essential nutrient in the leaves of *Amaranthus spinosus* was estimated with spectrophotometric method using 1, 10-phenanthroline[47]. The result showed that the leaves of *Amaranthus spinosus* contained a good amount of iron. (Table-6.2)

CHAPTER SIX

ANTIMICROBIAL ACTIVITY

6. MICROBIAL ACTIVITY

6.1 METHODOLOGY:

Disc diffusion method[37-40] is used for anti-bacterial activity test. In this method dried filter paper disk (6mm in diameter) containing the test materials are placed on Mueller Hinton agar plates seeded with the test organisms. These plates are then incubated at 37^0C for 24 hours to allow growth of the organisms. If the test material has any antibacterial activity, it will inhibit the growth of the microorganisms and a clear zone of inhibition will be visualized surrounded the disc. The antibacterial activity of the plant sample is determined by measuring the diameter of the zone of inhibition in mm. In this method diffusion of the test material occurs according to the physical laws that control the diffusion of molecules through an agar gel. As a result there is a gradual decrease of the test plant material concentration in the agar gel surrounding each disc. Well method can also be used for determining antibacterial activity. In this method wells are made in the plate which is about 6mm in diameter. Test materials are then added to these wells using appropriate solvent. Then the plates are incubated at 37^0C for 24 hours. After incubation, the zone of inhibition around the wells is observed.

6.2 Test microorganisms used for the study:

The bacterial strains used in the sensitivity test are listed in the following table:

Gram positive	Gram negative
Bacillus cereus	*Salmonella typhi*
Staphylococcus aureces	*Shigella dysentery*
	Vibrio Cholera
	Pseudomonas aeruginosa
	Escherichia coli

6.3 EXPERIMENTAL:

Muller Hinton agar media and nutrient agar media37, 38, 39, 40 were used for testing the sensitivity of the bacteria to the test materials and for preparative plate's cultures. The composition of these agar media are presented bellow-

Nutrient agar media		Mueller Hinton agar media	
Peptone	---- 5g/L	Peptone	----17.5 g/L
Sodium chloride	---- 5g/L	Beef infusion solid	----2.0 g/L
Beef extract	---- 3g/L	Starch	----1.5 g/L
Agar	---- 15g/L	Agar	----17.0 g/L
Final pH	---- 7.4±0.2 (at 25^{0}C)	Final pH	---- 7.3±0.2 (at 25^{0}C)

The media were prepared and sterilized by autoclaving at 121^{0}C for one hour. After sterilization these media were poured in sterile Petri dishes and solidified.

6.3.1 Preparation of the sample discs:

Crude extracts of pet ether was dissolved in sufficient amount of pet-ether. 30 µl of the solution was applied on sterile filter paper discs (to mm in diameter) left for sufficient time for completing evaporation of the solvent. The amount of plant extract within the disc was approximately.

Control discs using only the respective solvents were prepared in a similar way.

6.3.2 Inoculation and Inculation:

The test organisms were collected from ICDDRB. These organisms were transferred to the nutrient agar media from the supplied pure culture with the help of an inoculation loop. The inoculated media were then incubated at 37^0C for 24 hours for the growth of the microorganisms. These fresh cultures were used in the sensitivity test as inculum.

Cell suspensions of the test organisms were prepared in normal saline (0.85% Nacl) and adjusted as equal to a density of a Me Farland 0.5 standard at 625 nm is 0.08-0.10. When the proper density was achieved a sterile cotton swab was submerged in the suspension, lifted out of the suspension and excess liquid was removed by pressing and rotating the swab against the wall of the tube, just above the fluid level.

The swab was then used to inoculate the entire surface of the respective Mueller Hinton agar media three times, rotating the plates 60^0 between each inoculation. The inoculated plates wee allowed to dry (usually taking only few minutes but no longer than 15 minutes) before the prepared discs were placed on the plate. The prepared discs containing the test materials were placed on the plate using a sterile forceps. The control discs containing only the solvents were also placed on the same plate. The plates containing the discs were incubated at 37^0C for 24 hours in an inverted position in an incubator.

6.3.3 Estimation of susceptibility of the bacteria-

After over night incubation the plates were examined and the diameters at the zone of inhibition were measured in millimeters (mm).

6.4 RESULT:

Table 6.1. Susceptibility pattern of bacteria against petroleum ether extract.

PETR-ETHER EXTRACT:

Bacteria	Control	Sample (zone of inhibition in mm)
Salmonella typhi	Nil	Nil
Shigella dysentrae	Nil	Nil
Staph. Aureus	Nil	Nil
Bacillus cereus	Nil	Nil
Vibrio cholerae	Nil	Nil
Pseu. Aeruginosa	Nil	Nil
E. coli	Nil	Nil

6.5 DISCUSSION:

The result of the sensitivity test was summarized in above table. Seven standard strains of pathogenic bacteria used in this study were collected from ICDDRB. The control disc had no zones of inhibition and hence its data was not mentioned in the table. The entire gram positive and the gram-negative bacteria did not show sensitivity towards pet ether extract.

CHAPTER SEVEN

SUMMARY

SUMMARY

Bangladesh is rich with different types of plants. Most of these have medicinal importance. ***Amaranthus spinosus*** of the family Amaranthaceae is one of the medicinally important plant. It is used for various diseases like piles, renal dropsy, pneumonia, cough, kidney stone, skin diseases, snake-bite, gonorrhea, dysentery, etc.

Hormones, sterols, saponins, terpenoids, flavonoids, saponins, aminoacids, phenols, alcohols and ketones types of compounds have been isolated from these plants by different workers. More investigation and thorough study of this plant may open a new chapter towards the significant role of the plant for the human well beings. With this end in view, the phytochemical studies of the plant *Amaranthus spinosus* have been undertaken. The petroleum ether (b.p 40-60^{0}C)) ethyl acetate and chloroform extract have been investigated in this project.

The plants were collected from the Pabna district. The leaves are separated from the stems. The leaves, stems and roots are dried at 400^{0}C in an oven. The dried materials were made powder in a grinding machine.

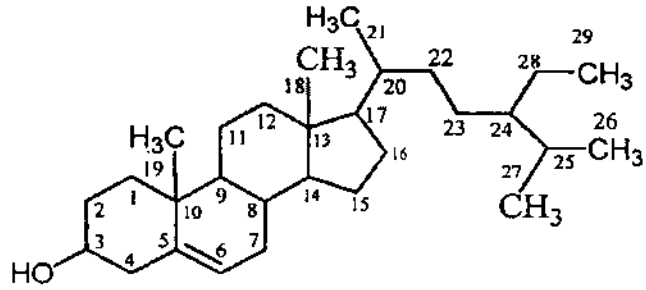
1) The root and stems powder were extracted with chloroform followed by ethyl acetate using a soxhlet apparatus. The chloroform extract was concentrated to a dry mass and this extract was subjected to VLC for separation of different compounds.

TLC analysis of the Chloroform extract showed several spots under UV light, in iodine chamber and vanillin-sulphuric acid spray on TLC plate.

The eluates from VLC of chloroform extract were mentioned by TLC and the fractions of similar behaviours were combined together and marked as T_1, T_2, T_3, T_4, T_5, T_6, T_7, T_8, T_9, T_{10}, T_{11}, T_{12}, T_{13}, T_{14}, T_{15}, T_{16}, T_{17}, T_{18}, T_{19}, T_{20}, T_{21}, T_{22}.

Among them TLC analysis of T_{11}, T_{13} and T_{14} were single compound. Fraction T_{11} (~50 ml) T_{13} and T_{14} were kept separately undisturbed for several days.

From T_{11}, T_{13} and T_{14} fractions a while solid were obtained which was designated as A_1, A_2 and A_3. The TLC study of showed a single spot (R_f value 0.40 in 6 : 4 DCM and pet-ether) A_1 had important frequencies of absorption at 3450,2900, 1660, 1480 cm^{-1}. After IR, ^{1}H-NMR and ^{13}C-NMR spectral analysis. The structure of the compound A_1 was designated as stigmast-5-en-3-β-ol. (β-sitosterol),



The analysis of A_2 and A_3 were not performed due to very small amount .

2) The dried and powdered leaves (850g) were extracted with petroleum ether. After drying petroleum ether extract, a solid mass (8.95g) was obtained. The dried extract was fractionated by VLC (Vacuum liquid column chromatography) using TLC grade silica gel as adsorbent and eluted with petroleum ether (401-60^{0}C), dichloromethane and methanol with increasing polarity. Fifteen fractions were obtained and these were designated as fractions C_1, C_2, C_3 .... C_{15}.

The fraction C_1 was concentrated and left over night while a needle shaped white crystal substance V_1 (19mg) settled at the bottom of the conical flask.
This substance was again dissolved in petroleum ether and its TLC studies showed that it was a single compound with R_f value (0.5).

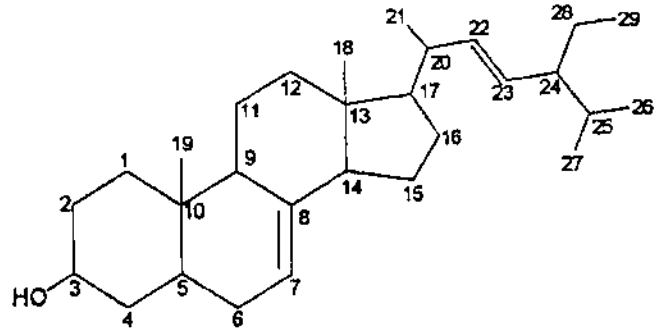
The fraction C_9 was concentrated and was allowed to stand at room temperature while off-white needle shaped crystals V_2 (26mg) was obtained with R_f value (0.55). Its TLC behavior also showed that it was a single compound.

Compound V_1 was characterized by IR, ^{1}H- and ^{13}C-NMR spectral studies as (22E) Stigmast-7, 22-dien-3β-ol (spinasterol) where the ^{13}C-NMR spectrum showed the presence of twenty-nine (29) carbon signals. Among these four (4) of them were assignable to sp^2 carbons, nine (9) signals to methylene carbons, six (6) signals to methyl carbons, eight (8) signals to methane carbons and two (2) signals for quaternary carbons.

Based on the IR, ^{1}H- and ^{13}C-NMR spectroscopic data, the compound V_1 has been proposed to have the following structure.

IR spectrum showed a broad absorption band at 3450 cm^{-1} assignable to hydrogen bonded hydroxyl group (-OH). The bands at 1430, 1350 together with the bands at 2950 cm^{-1} and 2850 cm^{-1} was due to the presence of $-CH_2$ and $-CH_3$ group respectively. The absorption band at 1050 cm^{-1} was due to presence of C-O stretching for alcoholic group. IR spectrum of V_2 is similar to 1-hexanol.

The ^{1}H-NMR spectrum showed a downfield broadened singlet at δ 4.061 ppm suggestive of the presence of hydrogen bonded primary alcoholic proton[9]. Triplet at δ 1.603 ppm is due to methylene protons, which attach to hydroxyl and methylene group ($-CH_2-$ CH_2- OH). The single broadened absorption peak at 1.245 ppm for the protons of seventeen- methylene group, which have similar chemical shift and same coupling constant in long chain[44]. The spectrum showed upfield triplet at 0.871 ppm for the methyl group, which attach to methylene group[44].



The ^{13}C-NMR spectral analysis of V_2 showed signal at 64.482 was suggestive of a methylene carbon being attached to a hydroxyl group[34]. The upfield signal (Fig:

3.23 & 3.24) at 14.137 indicated the presence of methyl carbon. The signals at 22.719- 34.462 for seventenn- methylene carbon, which were existing in long chain of primary alcohol[34]. by DEPT experiment, methyl and methylene carbons were fixed (Fig: 3.25).

The EI^+ mass spectra of the compound V_2 showed m/z 285 (M+1) indicating its molecular formula $\mathbf{C_{19}H_{40}O}$. The melting point of V_1 was compared with reported melting point of 1-Nonadecanol and it was same. The IR, ^{1}H-NMR, ^{13}C-NMR mass spectral analysis and melting point established the identity of compound V_2 as 1-Nonadecanol which has been proposed to have the following structure.

$\mathbf{CH_3 - [-CH_2 -]_{17} - CH_2 - OH}$

The fractions C_2, C_3 ..... C_8 and C_{10}, C_{11} .... C_{14} were mixture of compounds. So they were not analyzed further.

From this study, the presence of two compounds --- spinasterol and 1-Nonadecanol was characterized by the chemical and spectroscopic analysis from the plant *Amaranthus spinosus*.

3) The leaves powder was extracted with petroleum ether (b.p. 40 - 60^{0}C) followed by ethyl acetate (EA) using a soxhlet apparatus. The EA extract was concentrated to a dry mass. The EA extract was subjected to VLC for separation of different compounds.

The eluates from VLC of ethyl acetate extract were monitored by TLC. The elutes of ethyl- acetate extract were ten tractions marked as E_1, E_2, E_3 ..... E_{10}. The similar fractions were combined together by screening with TLC and these were marked as F_1, F_2, F_3, F_4 and F_5.

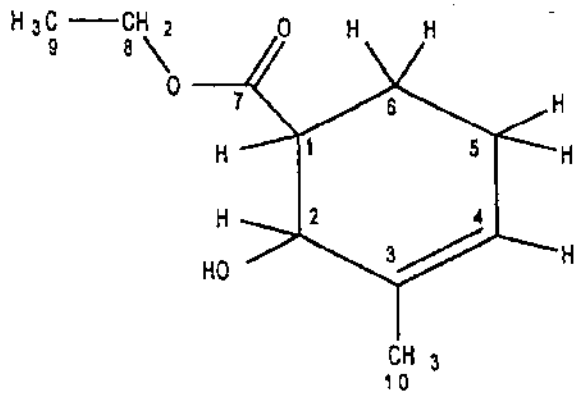
Fraction F_1, F_3 and F_5 were analyzed but no satisfactory results were obtained. The fractions F_2 and F_4 was analyzed.

spectrum of compound M_1 exhibited the frequency of absorption at, 3420, 2930, 2850, 1725, 1450, 1375, 1250 - 1050 and 720 cm^{-1}.

The compound M_3 was isolated from the fraction F_4 obtained upon VLC of ethyl acetate extract of the leaves of *Amaranthus spinosus* followed by preparative TLC.

Its TLC showed and elongated single spot (R_f value = 0.62; petroleum ether : dichloromethane = 4 : 1; silica gel). Upon exposure to iodine vapour a brown coloured spot was appeared. The spot turned into violet colour on spraying with vanillin-sulphuric acid followed by heating in an oven at 110^0C for 10 minutes. The elongation of the spot is though to be indicative of a compound with a polar part undergoing strong interaction with silica gel and remains at the bottom of the elongated spot while the non-polar part is moved by solvent towards the top of the spot resulting in its elongation (tailing).

Interpretation of IR, ^{1}H-NMR and ^{13}C data for M_3 should that a minor compound was mixed with major compound. On the basis of spectral data the structure of the major compound was tentatively proposed as Ethyl 2-hydroxy-3-methyl cyclohex-3-ene carboxylate.



The spectral data for minor compound supported that it was an ester with a large alkyl chain.

Its complete structure determination needs its purification, which could not be done at this moment.

4. Iron content of the plant was determined spectorphotometrically using 1,10- phenanthroline and the results showed that the iron contrent in *Amarantus spinosus* was 6.7 mg/100g dry powder. The ash content of *Amarantus spinosus* was determined and this was found to be 44.17%.

5. For antimicrobial test of petroleum ether extract, I types of bacteria and five types of fungi were selected. All these experiments were carried out by disk diffusion method. All of the strain tested showed no sensitivity towards petroleum ether extract.

Although attempts have been taken to evaluate their phytochemical studies to explore their medicinal value but enough work has not been done on the species.

Therefore, the present study has been undertaken to-

(a) Isolate and characterize different phytochemicals

(b) Evalute its nutrictional status and food values (determination of iron content)

(c) Study the anti microbial activity of different extract of the plant and hence to explore its potentiality (if any) in the field of medicine.

CHAPTER EIGHT

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