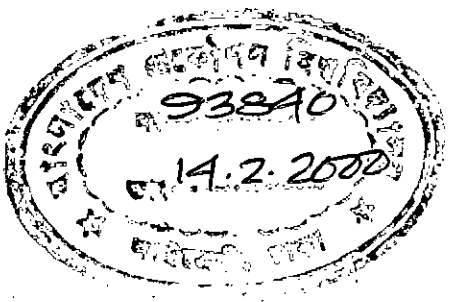
CHEMICAL INVESTIGATION ON THE STEMS OF POTKA (*GREWIA MICROCOS* LINN.) AND SHETODRONE (*LEUCAS ZEYLANICA* LINN.)

A DISSERTATION
SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF PHILOSOPHY IN CHEMISTRY



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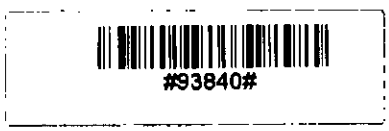

DECLARATION

I hereby declare that the whole of the work of this thesis has been carried out by myself in the Organic Research Laboratory of the Chemistry Department, BUET, Dhaka under the joint supervision of Dr.A.K.M. Matior Rahman, Associate professor in Chemistry, BUET, Dhaka and Professor Dr. Md. Abdul Quader of the Chemistry Department of Dhaka University, Dhaka during the period starting from July, 1997 to September, 1998. I, furthermore, declare that this work has not been submitted in part or full any where else for a degree or diploma. Any source of information in connection with this thesis has been duly acknowledged and all quotation has marked by quotation marks.

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বাংলাদেশ প্রকৌশল বিশ্ববিদ্যালয়

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THESIS ACCEPTANCE LETTER

We hereby recommend thesis entitled " Chemical investigations on the stems of potka (*Grewia microcos*) and Shetodrone (*Leucas zeylanica*) " presented by Parvin Begum , Roll No. 9403006 F, Registration No. 9403006, session 1993-94-95 to accept as partial fulfilment of the requirements for the degree of Master of Philosophy (M.Phil) on 4th October, 1998.

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SUMMARY

Leucas zeylanica (Labiatae) and *Grewia microcos* (Tiliaceae) grow all over Bangladesh. *Leucas zeylanica* (Labiatae) is locally known as "Shetodrone" and *Grewia microcos* (Tiliaceae) is popularly known as "Potka". Both these plants are medicinally important. *Leucas zeylanica* is used in the countryside for the treatment of cold, headache, snake-bite, ulcer and skin diseases (Kirtikar and Basu, 1980). On the other hand, *Grewia microcos* is taken by the patients for the remedy of eczema, small pox, typhoid fever, dysentery and ulcer (Kirtikar and Basu, 1980).

A literature survey revealed that a lot of work have been carried out on many species of both the genera Leucas and Grewia. But there appears no report of any work on both these plants. This fact has prompted us to investigate these plants thoroughly.

Leucas Zeylanica was collected from Dinajpur and *Grewia microcos* was procured from the bushy area of Jahangir Nagar University campus, Savar, Dhaka. The stems of the individual plants were separated manually, dried in shade, powdered, extracted with pet ether, EtOAc, MeOH and 80% EtOH successively.

The extracts of *Leucas Zeylanica* yielded LZFA, LZ1, LZ2, LZ3, LZ4, LZ5 and LZW.

LZFA was identified as a mixture of fatty acid esters. LZ1 was characterized as stimastan-7,22-di-ene-3-β-ol (22). LZ2 was identified as a new compound, 28-epoxy-stimaststan-7,22-di-ene-3-β-O-glucopyranoside (23). Also its acetyl derivative was prepared and identified.

LZ3 was identified as a stereoisomer of the compound LZ2 (23). LZ4 has been established as a 2-hydroxy-dicyclo-2,5-di-ene-7,8-dimethyl-3-O-β-D-glucopyranoside (24). This compound is also a new one. LZ5 was not conclusively identified but assumed to be a halogenated compound. LZW was examined by paper chromatography, and its alditol acetate derivative was analyzed by GLC. The results confirmed the presence of glucose, galactose, arabinose and xylose.

The individual extracts of *Grewia microcos* were concentrated and subjected to chromatography (CC, VLC and PTLC) while the compounds GMP1, GMFA, GME2 and GMM3 were isolated in pure form. Their IR spectra were recorded and analyzed to their identification . The compound GMP1 has been assumed as a triterpenoid carboxylic acid type compound on the basis of its IR spectrum and its NMR spectrum (60 MHz).

The techniques IR, ^{1}H-NMR, ^{13}C NMR, 2D NMR and EIMS have been extensively used for the characterization of the compounds.

Significance of the compounds has been discussed to focus their medicinal importance and their chemotaxonomic value.

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1.0 : CHAPTER 1

1.1.0 : INTRODUCTION :

The herb *Leucas Zeylanica Linn.* (Bengali name : Shetodrone) of the family *Labiatae* and the shrub *Grewia microcos linn.* (Bengali name : Potka or Asar) of the family *Tiliaceae* grow all over Bangladesh. For long the Ayurvedas, the Unanis and the herbal medical practitioners of our country have been successfully using different parts of these plants as remedy to various ailments. They along with other plants of their families are also used extensively as herbal medicines in different countries of South-East Asia including China. In spite of their wide medicinal appliances, these two plants did not receive any attention of the scientists at all to isolate and characterize their active principles that are effective against various ailments . Hence keen interest arose to study them phytochemically. A brief botanical and chemical information regarding these plants are given below.

1.1.1.0 : THE PLANT FAMILY *LABIATAE* :

The plant family *Labiatae* consists of 200 genera comprising of 3000 species. They are chiefly Asiatic and African (Kirtikar and Basu, 1980). They are widely distributed all over the Bangladesh, India, Burma, Sri-Lanka, Malaya, Java and China (Kirtikar and Basu, 1980) .

1.1.1.1 : *LABIATAE* SPECIES GROWING IN BANGLADESH :

The genus leucas belongs to the family *Labiatae*. It consists of nearly 100 species of which only five are widely distributed in different areas of Bangladesh (Kirtikar and Basu, 1980). These are listed in table-1.

Table 1 : *LABIATAE* SPECIES AVAILABLE IN BANGLADESH

Native name	Scientific name	
	Genus	Species
Dulfi, Dandakolos, Shetodrone	Leucas	Aspera
Bara Dandakolos, Dhrupy	Leucas	Cephalotes
Bara Halkusha	Leucas	Involucrata
Gaochia, Chhoto Halkhusha	Leucas	Lavaendulaefolia
Shetodrone, Kusha	Leucas	Zeylanica

1.1.1.2 : BOTANICAL CHARACTERISTICS OF *LEUCAS ZEYLANICA (LINN)*

Leucas Zeylanica (Linn.) is an annual erect evergreen herb.It is locally known as Shetodrone. Its morphological features as described by Kirtikar and Basu, 1980 are briefly discussed below. Figure 1.1 is a matured plant of *Leucas Zeylanica (Linn.)* in its growing stage.

Nature : *Leucas Zeylanica Linn. (Shetodrone)* is a herb. It is a green plant with many branches from very near the base ; **Leaf** : Leaves Subsessile , variable , 2.5-7.5 by 0.8.1.3 cm. , linear or

linear-lanceolate , obtuse , the margins often slightly recurved , entire or serrulate , hispid on both sides , ciliate with often bulbous based hairs , base tapering ; midrib conspicuous beneath , hairy ; **Flower** : Flowers sessile or nearly so, usually in terminal whorls 1.3.2 cm. diam. ; bracts 6 mm. long , linear , spinous-pointed , ciliate with long bulbous-based hairs ; **Calyx** : Calyx 6 mm. long , turbinate , slightly curved , the lower part glabrous or slightly pubescent outside , the upper part with a few long scattered spreading hairs ; mouth broad oblique , the upper part projecting forward, villous, the villi shorter than the calyx teeth ; upper tooth of calyx longer than the others , 1.25 mm. long , triangular , acute, the other teeth subequal, about 0.85 mm. long, subulate ; **Corolla** : Corolla white, 2 cm. long ; tube 6 mm. long , densely white-wooly, the middle lobe broadly obovate, cuneate, rounded or sub-truncate, emarginate, the lateral lobes very small; **Nutlets** : Nutlets 3 mm. long, obovoid-oblong, rounded or truncate at the apex , the inner face sharply angular, the dorsal face rounded , smooth , brown ; **Distribution** : It is widely distributed all over Bangladesh , India , Burma , Sri-Lanka , Malaya , Java and China (**Kirtikar and Basu**, 1980). In Bangladesh it grows mainly in the waste places and road sides but specially in the Districts of Dhaka Comilla, Sylhet, Mymensingh, Dinajpur, Netrakona, Chittagong and Chittagong Hill Tracts (**Ghani**, 1998).

1.1.1.3 : MEDICINAL SIGNIFICANCE OF ***LEUCAS ZEYLANICA LINN.*** AND SOME OTHER SPECIES OF THE GENUS ***LEUCAS*** :

Juices from different parts (leaves, roots and stems) of the ***Leucas Zeylanica Linn.*** have reputation as a potent remedy of

various ailments such as scabies and other skin diseases, headache and colds. Decoction of leaf is applied to ulcers of the nose (Ghani, 1980). In Sri-Lanka, the leaves are bruised and a teaspoonful of the juice given, which is sniffed up as a remedy in snake-bite (Kirtikar and Basu, 1980).

Like *Leucas Zeylanica Linn.*, the *Leucas* species *Aspera Willd. and Chepaloties Roth. also* belonging to the family *Labiatae* grow abundantly in Bangladesh. It is reported that plants of *Aspera Willd.* are antipyretic and antidote to snake-venom. Its leaves are useful in chronic rheumatism. The juices from its various parts are antibacterial and extensively used for the treatment psoriasis, scabies and chronic skin eruptions. Flowers are used to get rid of cold (Ghani, 1998). Plant of *Chepaloties Roth.* is stimulant and diaphoretic. It is laxative, anthelmintic, useful in bronchitis, jaundice, inflammations, asthma, dyspepsia, paralysis and leucoma. Fresh juices are applied in scabies and eczema. Flowers are stimulant, aperient, diaphoretic, emmenagogue and expectorant, and a syrup is used as a remedy for colds and coughs (Kirtikar and Basu, 1980).

1.1.1.4 : CHEMICAL STUDIES ON *LEUCAS ZEYLANICA LINN.* AND OTHER SPECIES OF THE GENU *LEUCAS* (A LITERATURE REVIEW) :

From a thorough literature review it appears that no chemical investigation has been done on leaves, roots and

stems of Shetodrone, *Leucas Zeylanica Linn.* prior to this study. However, there are reports on isolation of multiple compounds from others species of the genus *Leucas* are available in the literature. The following are some of these reports :

Misra et al., (1992) reported presence of aliphatic ketols, 28-hydroxy pentatriacon-7-one, 7-hydroxydotriacontan-2-one and β-sitosterol from the shoots of *L. Aspera Willd.* The presence of 1-hydroxytetratriacontan-4-one, 32-methyl tetratriacontan-8-ol, and dotriacontanol has also been reported from the shoots of *L. Aspera Willd.* (Misra et al., 1992). Also according to Ghani, (1998) leaves of *L. Aspera Willd.* contains glucosides, tannins, saponins and sterols α-and β-sitosterol. Oleic, linoleic, palmitic stearic, Oleanolic and ursolic acids have been isolated from this plant. 5-acetoxytriacontane and β-sitosterol have also been isolated from this plant.

Hasan, (1991) reported the presence of diterpene fatty acid ester, trans-phytyl palmitate, n-hentriacontane, 1-dotriacontanol and phytol from the plant of *L. nutans*. Leucasin, 3-O-[β-D- glucopyranosyl (1-2) β-D-glucopyranosyl], 2α, 3β-dihydroxylup-20(29)-ene, lupeol, palmitate, sitosterol and stigmasterol were also found in *L. nutans* (Hasan, 1991).

Khalil, (1996) isolated three labdane-type diterpenes 3-ox-omarrobiin and a mixture of two related C-15 epimeric diterpenes

based on 9α, 13α, 15, 16-bisepoxy-15-hydroxy-3-oxo-labdan-6β, 19-oxide, acylated flavone apigenin-7-o-[6"-o-(p-hydroxy-trans-cinnamoyl) glucoside] from *L. neufliseana*.

Singh, (1994) isolated triterpenoids , steroids and alkaloids from stems of *L.aspera, L.lavandulifolia, L. stricta* and *L. cephalotes*. Purba, (1985) also reported the isolation of acacetin and chrysoeriol from *L. lavandulifolia*.

Dina and Jana, (1987) the reported the isolation of campesterol, brassicasterol,cholesterol, β -sitosterol and stigmasterol from *L. lanata*. Singh et al., (1988) reported the presence of quercetin and kaempferol as the major flavonoids from EtOAC extract of leaves and fruits of *L. urticaefolia*.

Lamaty et al., (1994) reported the presence of essential oil, sesquiterpene, hydrocarbon, germacrene, β-caryophyllene and α-humulene while Mahato and Pal, (1986) isolated linifolioside defined as isopimara-8(14),15 diene-7-keto-3-o- α -L-ramnopyranosyl (1-2)-β D-glucopyranoside from the leaves of *L. deflexa*.

Flowers of *L. Chepaloties Roth*. contain a small quantity of an essential oil and an alkaloid. Alcoholic extract of the whole plant contain β-sitosterol, a glycoside of β-sitosterol and traces of an alkaloid. Seeds contain an oil rich in labellinic acid and its esters. An isopimarane rhamnoglucoside and linifolioside have been isolated from the plant of *L. Lavandulaefolia Rees* (Ghani, 1998)

The structures of β-sitosterol (1), Ursolic acid (2)

and Linifolioside (3) isolated from different species of the genus *Leucas* belonging to the family *Labiatae* are in the following page.

1.1.2.0 : THE PLANT FAMILY *TILIACEAE* :

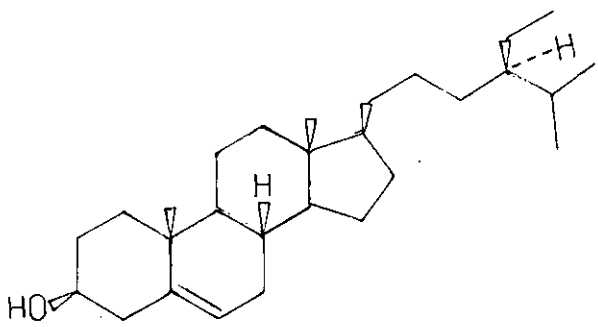
The family Tiliaceae consits of 35 genera comprising of 380 species--- tropical and temperate. They are widely distributed in South East Asia, Europe, Brazil, Africa and Australia (Kirtikar and Basu, 1980).

1.1.2.1 *TILIACEAE* SPECIES GROWING IN BANGLADESH :

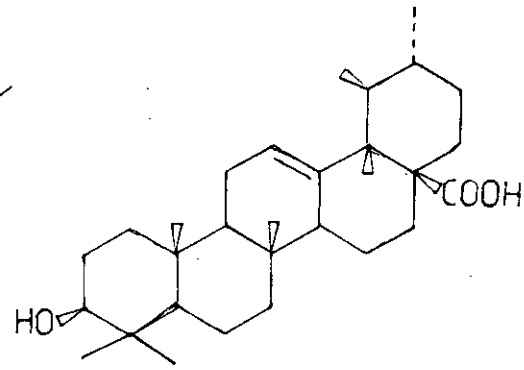
The genus *Grewia* is a member of the family *Tiliaceae*. It consists of 150 species (Kirtikar and Basu, 1980) which are widely distributed in Asia, Africa and Australia, especially tropical. The Grewia species available in Bangladesh are listed in table-2.

Table 2 : *TILIACEAE* SPECIES FOUND IN BANGLADESH :

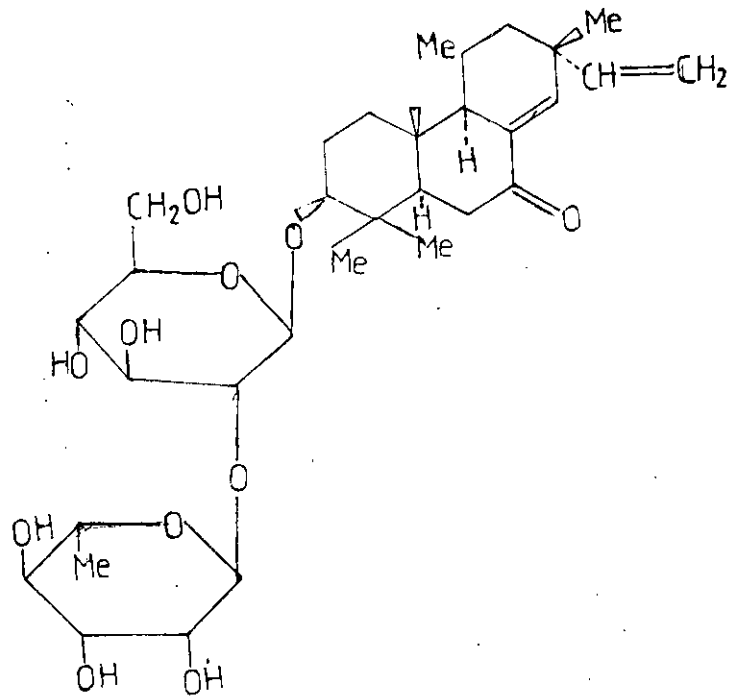
Native name	Scientific name	
	Genus	Species
Phalsa, Sakri (Beng.)	Grewia	Asiatica
Dhamani, phalsa	Grewia	Tiliaefolia
Asar, Potka	Grewia	Microcos
Dhamni, phalsa	Grewia	Subinequalis
-	Grewia	Sapida
-	Grewia	Rothii
-	Grewia	Micromacrophylla



β-sitosterol (1)



Ursolic acid (2)



Linifolioside (3)

Fig. 1.1 : A matured plant of *Leucas Zeylanica Linn.*

Fig. 1.2 : A sub-branch of *Grewia microcos Linn.*

1.1.2.2 : BOTANICAL CHARACTERISTICS OF ***GREWIA MICROCOS LINN.*** :

The local name of the shrub ***Grewia microcos Linn.*** is Potka or Asar. Its morphological characteristics as described by Kirtikar and Basu, 1980 are briefly described below. Figure 1/2 represents a sub-branch of the plant ***Grewia microcos Linn.***.

Nature : Potka or Asar, ***Grewia microcos Linn.*** is a shrub type of green plant, the young parts of which are stellately puescent. **Leaf** : Leaves 10-15 by 3.8-5.7 cm., elliptic-oblong, acuminate, glabrous, entire or slightly and irregularly toothed, base oblique, rounded or acute, 3-nerved, the nerves and veins prominent beneath ; petiols 6-10 mm. long ; stipules 6-13 mm. long, linear, acute. **FLOWER** : Flowers in terminal panicles ; buds subglobose or ovoid, grey-tomentose, 2 or 3 close together enclosed within an involucre of 6-8 oblong-lanceolate imbricate bracts ; pedicles short ; bracts of the pedicles subulate. Sepals 6 mm. long , obovate-oblong tomentose on both surfaces . Petals less than one third the length of the sepals, ovate, acute, pubescent at the base outside ; gland half as long as thwe petal, slightly ciliate. Torus short, lobed at the apex. **Ovary** : Ovary glabrous ; style much longer than the stamens, glabrous ; stima minute, befid. **FRUIT** : Fruit globose or slightly obovoid, about 10 mm. across, purplish, glabrous, wrinkled, mesocarp fibrous ; stone 1-celled. **Distribution** : It is widely distributed all over the Bangladesh, India, Burma,

Sri-Lanka, China and Java (**Kirtikar and Basu, 1980**). In Bangladesh it grows wild mainly in waste places and road sides but specially in the district of Dhaka, Chittagong and Chittagong Hill Tracts.

1.1.2.3 : MEDICINAL SIGNIFICANCE OF ***GREWIA MICROCOS LINN.*** AND SOME OTHER SPECIES OF THE GENUS ***GREWIA*** :

According to **Kirtikar and Basu** (1980) ***Grewia microcos Linn.*** is a much used medicinal plant of Bangladesh, India, Burma, Sri-Lanka, China and Java. It is given for indigestion, eczema and itch, small pox, typhoid fever, dysentery and syphilitic ulceration of mouth.

Like *Grewia microcos Linn.*, the *Grewia* species *Grewia tiliaefolia and Grewia asiatica Linn.* belonging to the same family *Tiliaceae* abundantly in Bangladesh. According to Ayurveda sources the bark of *G. tiliaefolia* is acrid with a sharp sweetish taste ; digestible , strengthening , aphrodisiac ; heats the body ; heals wounds ; cures "kapa," "vata," burning sensation, thirst, throat complaints, biliousness, cough, diseases of the nose, and of the blood. The fruit is sweet and cooling, slightly acrid ; removes "kapa" and "vata". The bark of the tree is also employed externally to remove the irritation from cow-itch. In the Konkan the bark, after removal of the tuber, is rubbed down with water, and the thick mucilage strained from it and given in 5-tola doses,

with 2 tolas of the flour of ***Pancium miliaceum*** (warri) as a remedy for dysentery. it is also said that the wood reduced to powder acts as an emetic , and is employed as an antidote to opium poisoning (**Kirtikar and Basu, 1980**).

The unripe fruit of the ***Grewia asiatica Linn.*** is bitter, acrid, sour ; removes 'vata", causes "kapa"; and biliousness. The ripe fruit is sweet, pleasant to the taste, and cooling ; digestible, tonic, aphrodisiac; allays thirst and burning sensation; removes "vata" and biliousness; cures inflammations, heart and blood disorders, fevers, and consumption. The fruit is good for the troubles of throat ; helps removal of the dead foetus. The bark cures bioliousness and "vata" ; removes urinary troubles, and burning in the vagina. The fruit is sour and sweet ; strengthens the chest and the heart ; relieves thirst and hiccough ; useful in diarrhoea and fevers. The root and bark are used in strangury, gleet, and gonorrhoea. An infusion of the bark is used as demulcent. The fruit is supposed to possess astringent, cooling and stomachic properties. The leaves are used as application to pustular eruptions, and the buds are also prescribed by the practitioners. The roots and barks are also used in rheumatism in some places.

1.1.2.4 : CHEMICAL STUDIES ON ***GREWIA MICROCOS LINN.*** AND OTHER SPECIES OF THE GENUS ***GREWIA*** (A LITERATURE REVIEW) :

From a thorough literature review it appears that in spite of its multiple medicinal appliances no chemical investigation has been done on the plant species ***Grewia microcos Linn.***. However, reports of phytochemical work on other species of the genus

Grewia are found in the literature. The following is a brief survey of the reports available on some of the *Grewia species*.

Ross et al., (1987) reported isolation of harman, harmarine, harmol, harmalol and harmaline. They also reported the presence of sucrose, glucose and galactose from *G. villosa* root. Bashir et al., (1986) isolated 19-hydroxyuvaol,quinovic acid and β-sitosterol-3-O-glycoside from roots of *G. villosa*.

Jaspers et al., (1986) isolated β-sitosterol, Lupeol and betulin as well as other terpene esters from the petroleum ether extracts and β-sitosterol-3-O-glycoside, herman, 6-methoxyharman and 6-hydroxyharman from methanol extract of *G. bicolor*. Mukherjee et al., (1981) reported the presence of triacontanol, tetratriacont-21-ol -12-one and β-sitosterol from the leaves of *G. populifolia*.

Ganguly et al., (1966) isolated β-sitosterol and stigmasterol from the seeds of *G. populifolia* while Sarkar et al.,(1959) isolated glucose, arabinose, proline, serine, glutamic acid,phenylalanine, isolucene, and lysine from the stem of *G. populifolia*.

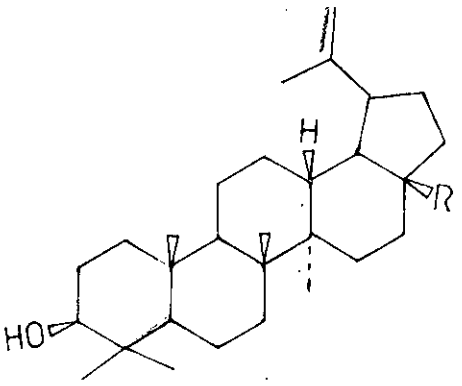
Bhagat et al., (1974) isolated lupeol and leucoanthocynidins from the root bark of *G. rothii*. Herzog et al., (1993) reported the presence of vitamin, carotene and mineral from the leaves of G. carpinifolia.

Prakash et al., (1979) isolated triacontan-1-ol, β-amyrin, β-sitosterol,lupenone,erythrodiol, α-amyrin and betulin from the stem-bark of *G.tenax*. They also reported the presence of β-amyrin, α-amyrin, β-sitosterol and erythrodiol from the stem bark of *G. flavescens*.

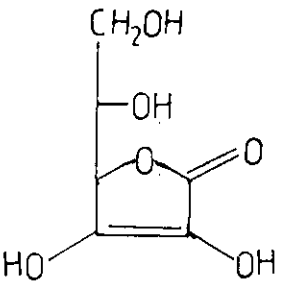
James et al.,(1983) isolated protein, fat, thiamin and ascorbic acid from *G. humilis* while Garg et al., (1990) isolated some alkaloids from *G. tenax*. Anjaneyulu et al., (1965) reported the presence of several known triterpenoids, steroids, coumarine, leucoanthocyanins , carbohydrates and alkaloids from a number of Indian species of the genus *Grewia* such as *G. tiliaefolia*.

Khurdiya et al., (1981) reported the isolation of 2-anthocyanin pigments, delphinidin-3-glucoside and cyanidin-3-glucoside from the fruit of *G. subinaequalis*. Hasnain and Rashda, (1992) isolated threonine from pulp, methionine from seeds, phosphoserine, serine and taurine from the juice of *G. asiatica*.

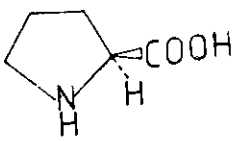
The structures of the compounds Betulin (4), Ascorbic acid (5), Proline (6), Phenyl-alanine (7), Glutamic acid (8), Isoleucine (9), Thiamine (10), Erythrodiol (11), Harmaline (12), Quinovic acid (13), α-amyrin (14) and β-amyrin (15) isolated from different species of the genus *Grewia* of the family *Tiliaceae* are given in the following two pages.



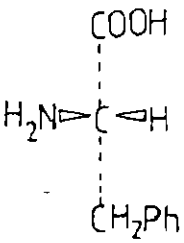
Betulin (4)



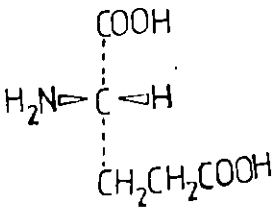
Ascorbic acid (5)



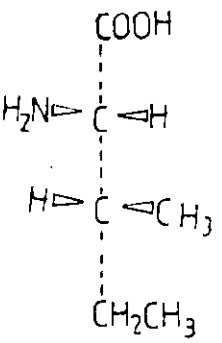
Proline (6)



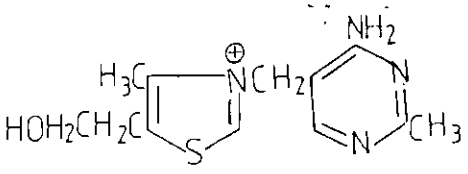
Phenyl-alanine (7)



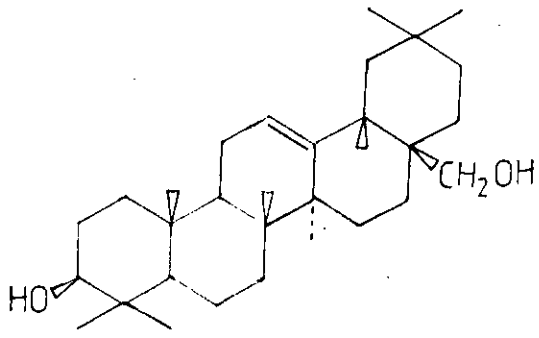
Glutamic acid (8)



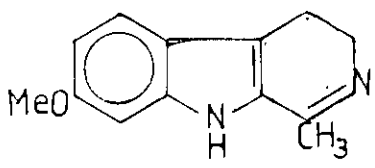
Isoleucine (9)



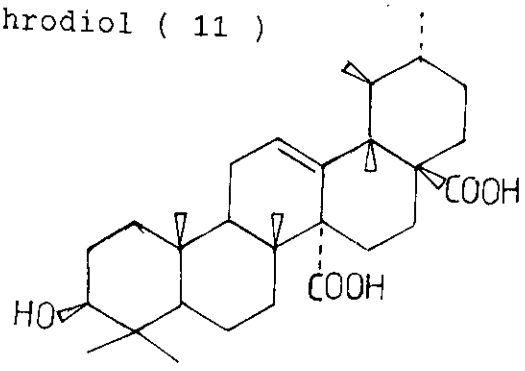
Thiamine (10)



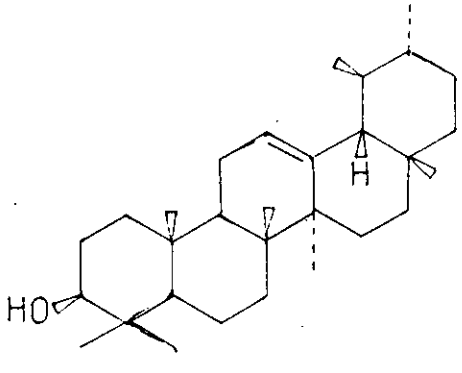
Erythrodiol (11)



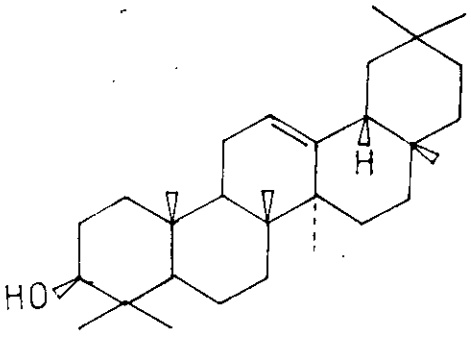
Harmaline (12)



Quinovic acid (13)



α-amyrin (14)



β-amyrin (15)

1.1.3.0 ECONOMIC IMPORTANCE OF *LEUCAS ZEYLANICA LINN.* AND *GREWIA MICROCOS LINN.* :

Medicinal plants are rich sources of bioactive compounds and thus serve as important raw materials for drug production. There are many countries in the world which earn a substantial amount of foreign currency by exporting medicinal plants as well as crude plant drugs. India and Thiland are two brilliant examples of such countries in the subcontinent. There are still other countries such as China, India and Pakistan which utilize their own medicinal plants for local manufacture of both Eastern and Western medicines and Pharmaceutical products. In contradiction Bangladesh in spite of having a large flora of medicinal plants every year imports a huge quantity of Pharmaceutical raw materials including medicinal plants and semi-processed plant products to feed its various drug manufacturing industries. According to herbal medicinal sources the herb *Leucas Zeylanica Linn.* and the shrub *Grewia microcos Linn.* have multiple medicinal appliances. Not only these two plants but also many other species of the two genuses *Leucas* and *Grewia* play a very important role in the field of herbal medicine. Although no report on chemical investigation of these two plants is available in the literature quite a large number of reports are available on the other species of these two genuses from different corners of the world. Plenty of compounds including various types of drugs e.g. alkaloids, steroids, cardiac glycosides, vitamins, minerals, amino acids etc. have been isolated or in some cases at least their

presence in these plants have been assured. The two plants ***Leucas Zeylanica Linn.*** and ***Grewia microcos Linn.*** along with their some other species grow abundantly in the jungles, bushes, waste places and also in the road sides of our country. Proper chemical investigation being done on them along with the other members of these families, the chemical compounds present in them will be known and then it will be possible to use them in the preparation of both herbal and western medicines. In view of the presence of a large number of chemical compounds or drugs in them, they might play a very important role in the economy of our country. But again derivation of economic benefits from them depends upon how these plants are used by our chemists, druggists, biochemists and pharmacists. However, once their chemical composition is known, the maximum economic benefit that can be derived from them is to utilize them as raw materials for indigenous drug industries, if not possible to export them in foreign countries at the present stage of our development.

1.1.4.0 : BIOGENESIS OF STEROIDS :

Steroids possessing C_{27}-C_{29} skeletons are not triterpenes having a C_{30} skeleton. But all the steroids have been derived from the same C_{30} precursor. Squaline (16) is derived from two farnesyl pp units which must be joined in the unusual " head to head manner"

According to the Sheme-1 the polycyclic structures can be

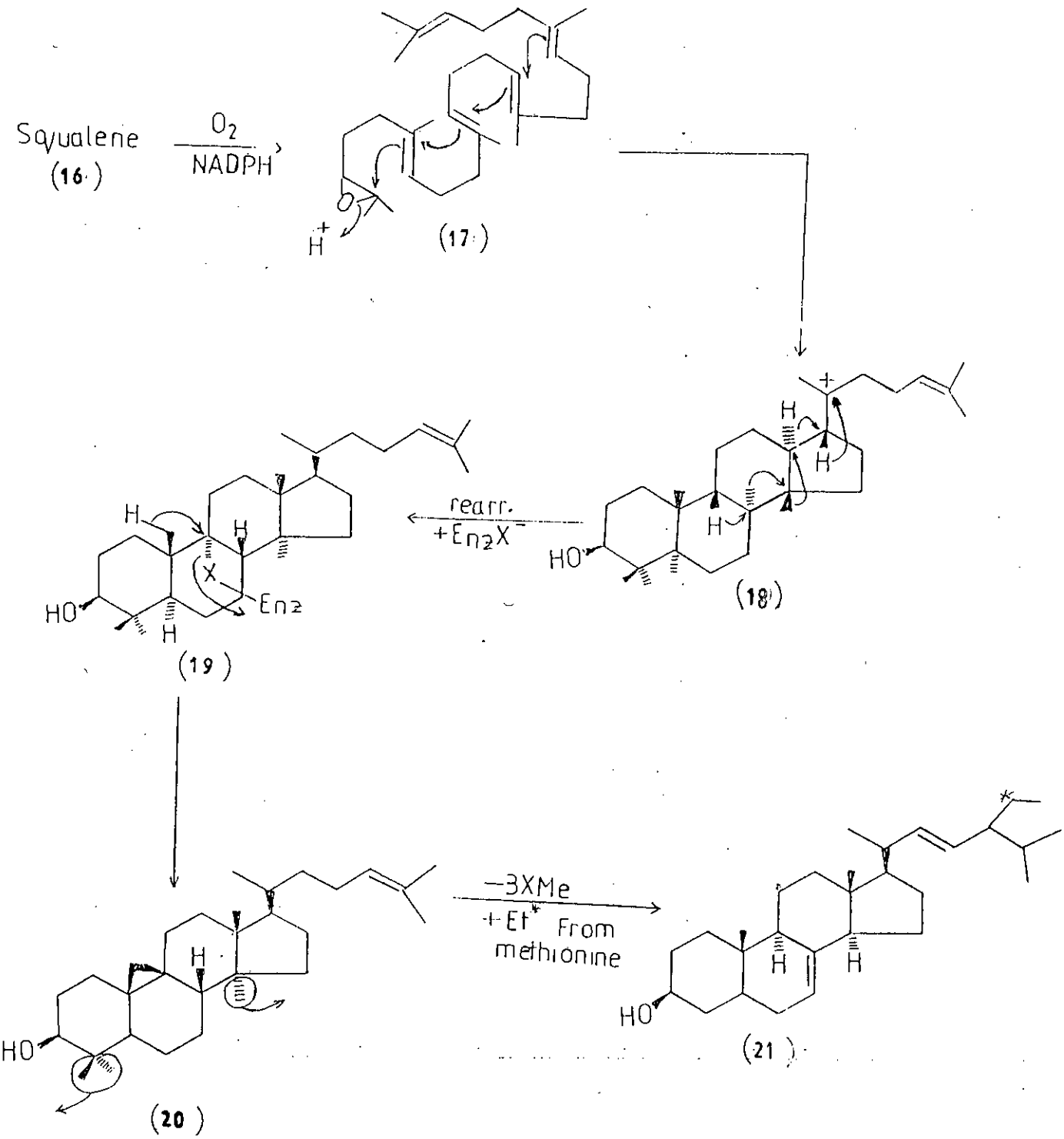
formed from squalene when squaline is folded (Pseudo chair and boat conformation) on enzyme surface. This is usually initiated by acid catalyzed ring opening of squaline monoepoxide (17) and probably occurs via a series of carbocationic intermediates. The initially formed cationic species (18) gives stigmasterol (21) through a series of reactions via formation of (19) and cycloartenol (20) (**Atkins**, 1987).

1.1.5.0 : BIOSYNTHESIS OF CARDIAC GLYCOSIDES :

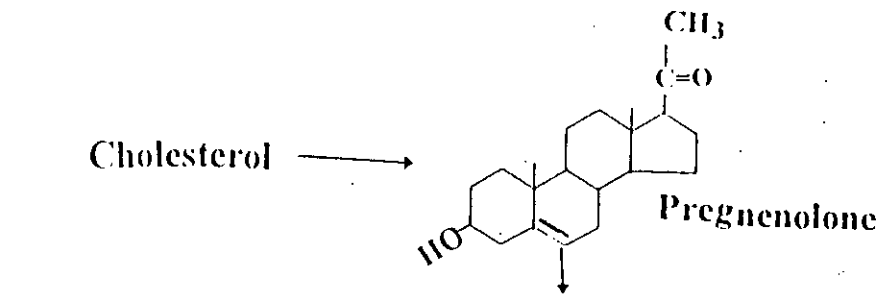
Aglycones of the cardiac glycosides are derived from mevalonic acid but the final molecules arise from a condensation of C_{21} steroid with a C_2 unit (the source of C_{22} and C_{23}). Bufadenolides are condensation products of C_{21} steroid and a C_3 unit (Scheme-2).

Progesterone which is formed with cardiac glycosides, in ***Digitalis lanta*** as a result of feeding pregnenolone is itself a precursor of the cardiac glycosides. Work involving ***Stophanthus kombe***, on the intermediates between progesterone and the cardenolide affords evidence consistent with the pathway (scheme-3).

Some of these transformation have also been demonstrated in ***Digitalis purpurea*** cultures and the mechanism of the 14β-hydroxyl-ation continues to receive much attention (Bennet et al., 1968 ; Furuya et al., 1973 ; Rees et al., 1976).

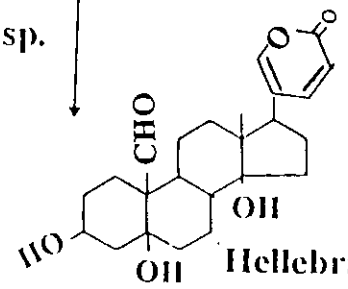


Scheme [1] : Possible biogenesis of Stigmasterol [21]

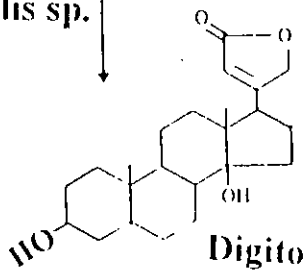


C_3Unit
Helleborus sp.

C_2Unit
Digitalis sp.

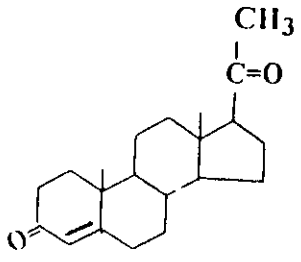


Hellebrigenin
(A bufadenolide)

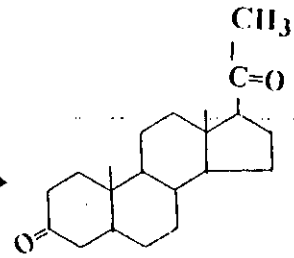


Digitoxigenin
(A cardenolide)

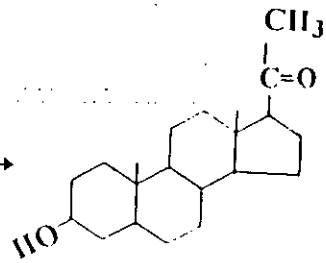
Scheme 2: Formation of Aglycones of Cardiac glycosides from C_{21} Steroid

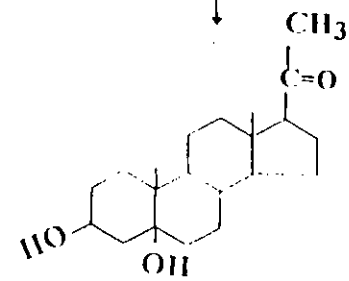


Progesterone

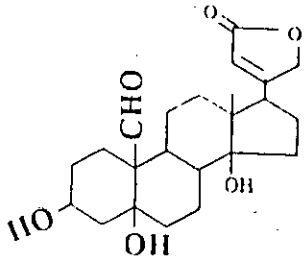


5β–Pregnenolone





C_2Unit



Strophanthidin

Scheme 3: Suggested intermediates in the metabolism of Progesterone to Cardiac glycosides

1.1.6.0 : CHEMISTRY OF CARDIAC GLYCOSIDES AND ESSENTIAL FIGURE FOR CARDIAC ACTIVITY :

Chemistry of cardiac genin :

Many of the plants now known to contain cardiac or cardiotonic glycosides have long been used either as arrow poisons or drugs. They are used therapeutically to strengthen a weakened heart and they allow the heart to function more effectively. The therapeutic efficiency depends both on the structure of the genin and the type and number of sugar units to which it is attached (Trease and Evans, 1980).

The genins have a steroidal structure and are chemically related to bile acids, Vitamin-D and sex hormones and to the venoms secreted by parotid glands and skin of certain toads (Templeton, 1969).

Two types of genin may be distinguished according to whether there is a five or six membered lactone ring. These types are known respectively as cardenolides, e.g. digitoxigenin and bufadenolides, e.g. Scillarenin. The following figures indicate their structures and numbering. Also some typical genins and the groups in the various positions are given in the Table-3.
The stereochemistry of these molecules is important for therapeutic activity :

(1) The hydroxyl at C_3 (the glucoside forming group) is β-oriented
(2) The *cis* configuration of the A-B ring.
(3) The β-orientation of C_{14} hydroxyl.

(4) The unsaturated lactone ring at C_{17} are all important for cardioactivity.

Uzarin, a glycoside of the Asclepiaaceae has all the essential features for activity except that the A/B ring has a trans configuration rendering it practically noncardio active.

Table-3 SOME TYPICAL GENINS AND THE GROUPS IN THE POSITIONS SHOWN.

Genins	1	3	5	10	11	12	14	16
Digitoxigenin	-	OH	H	CH_3	-	-	OH	-
Gitoxigenin	-	OH	H	CH_3	-	-	OH	OH
Digoxigenin	-	OH	H	CH_3	-	OH	OH	-
Strophanthidin	-	OH	OH	CH_3	-	-	OH	-
Ouabagenin	OH	OH	OH	CH_2OH	OH	-	OH	-
Scillaridin A	-	OH	H	CH_3	-	-	OH	-

Chemistry of sugar components :

Although fundamental pharmacological activity of the cardiac glycosides resides in the aglycone portion, the activity is considerably modified by the sugar moiety. The low solubility of aglycones is increased by union with sugars and the presence of later increases the power of fixation to heart muscle. The genin may be attached to from one to four monosaccharide molecules which have the structures of di-, tri- or tetrasaccharides. Thus from a single genin, one may have a series of tetra, tri-, di- or monoglycosides. Under suitable condition of hydrolysis , e.g. by specific enzyme, the sugar units may be progressively removed from

the genin. Some of the sugar units found in cardiac glycosides (Figure 3) are normal such as the hexose glucose and the methyl pentose, rhamnose, others are deoxy sugar, containing less than normal amount of oxygen, which have so far not been found in nature except in cardiac glycosides. Among these names of the following rare sugars may be mentioned.

Antianose (deoxyglucose), digitalose (a methyl ether of antianose), thevetose or cerberose (a methyl ether of deoxyglucose), digitoxose (a deoxyhexose) and cymarose (a methyl ether of digitoxose).

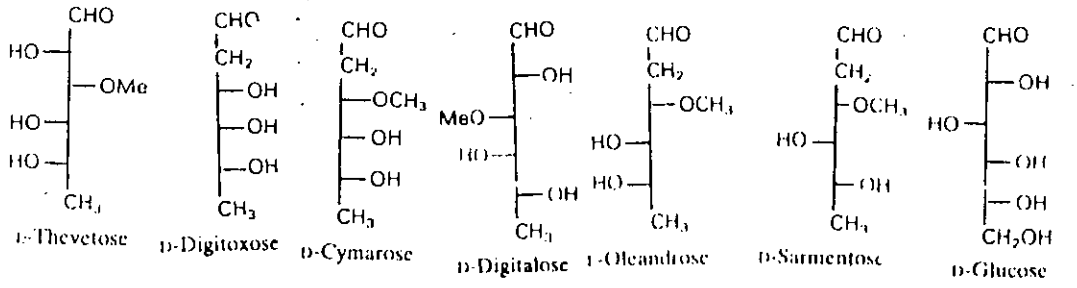


Fig.3 : Sugar units found in cardiac glycosides

1.1.7.0 : PURPOSE OF THE WORK :

It is evident from literature that many species of the genus Lucas of Labiatae family and genus Grewia of Tiliaceae

family have been thoroughly investigated and various types of compounds e.g. fatty acids, terpenoids, steroids, Amino acids, sugars etc. have been isolated from them. However, no report could be found on ***Leucas Zeylanica Linn.*** and ***Grewia microcos Linn.***. Hence chemical investigations were undertaken on these two plants.

1.1.8.0 : AIM OF THE PRESENT WORK :

The present project was designed to isolate pure compounds and determine the structures of the isolated compounds and if possible to study their biological activities. The project consist of the following steps :

(A) Extraction of the stems of the two plants ***Leucas Zeylanica Linn.*** and ***Grewia microcos Linn.*** with organic solvents of increasing polarity e.g. Petroleum ether (Pet ether, b.p 60-80^0), Ethyl acetate (EtOAC) and Methanol (MeOH).

(B) Fractionation of crude extracts by Column Chromatography (CC) , Vacuum liquid chromatography (VLC).

(C) Isolation and purification of pure compounds from the CC and VLC fractionated parts of the extracts.

(D) Determination of the structures of the isolated compounds with the help of chemical and physical methods e.g. UV, IR, ^{1}H-NMR, ^{13}C- NMR , Mass spectroscopy and two dimensional NMR (2D NMR) techniques, where necessary.

(E) If possible to study the ***in vitro*** antibacterial activity of the crude extracts and the pure compounds isolated from the extracts.

2.0 : CHAPTER 2

2.1.0 : METHODOLOGY :

2.1.1.0 : GENERAL METHODS :

2.1.1.1 : PREPARATION OF EXTRACTS :

The plant`s powder was extracted separately and exhaustively in a soxhlet apparatus with organic solvents of increasing polarity e.g. with petroleum ether (pet ether b.p. 60-80^0 C), Ethyl acetate (EtOAC) and finally with Methanol (MeoH). The methanol extract free residues were extracted with 80 % Ethanol (EtOH) to extract free sugars and amino acids if any.

2.1.1.2 : EVAPORATION AND CONCENTRATION :

All evaporations and concentrations were carried out under reduced pressure using rotary vacuum evaporator at bath temperature not exceeding 40^0 C.

2.1.1.3 : DETERMINATION OF MELTING POINTS :

All melting points were taken in a Fisher John`s Electrothermal melting point apparatus (Model no. 1A 9000). The heating was done carefully so as to maintain a steady temperature.

2.1.1.4 : CRYSTALLIZATION AND FRACTIONAL CRYSTALLIZATION :

In Organic Laboratory, the techniques of crystallization and fractional crystallization are frequently used for purification of

CC or VLC separated fractions. In the technique of crystallization usually a solvent is chosen in which the substance or the separated crude fraction is least soluble. The compound or the fraction is dissolved in a minimum volume of the solvent at an elevated temperature (filtered when necessary) and left undisturbed for crystallization or fractional crystallization. Sometimes some crystals are added as seed. In some cases, especially in case of fractional crystallization mixtures of solvents are used. The compound is dissolved in a suitable solvent and then a solvent in which the compound is insoluble or less soluble is added gradually until cloudiness is appeared and then it is left undisturbed for crystallization. When a batch of crystals are formed, the mother liquior is separated, concentrated and left for a 2nd batch of crystals.

2.1.1.5 : SOLVENTS AND REAGENTS :

All the solvents and reagent used in the experiments were procured either from E.Merk or B.D.H or Aldrich and were either meant for laboratory use or were of the analytical reagent grade. All the solvents were distilled before use. Before distillation pyridine was dried over phosphorus pentoxide and the distillate boiling at 115^{0}C was collected over potassium hydroxide pellets. Before use for acetylation pyridine was finally dried over molecular seive.

2.1.2.0 : CHROMATOGRAPHIC TECHNIQUES :

For separation of the crude extracts into individual pure compounds, various types of chromatographic technique were used e.g. Paper Chromatography (PC), Vacuum Liquid Chromatography (VLC), Thin Layer Chromatography (TLC) and Preparative Thin Layer Chromatography (PTLC) .

2.1.2.1 : PAPER CHROMATOGRAPHY :

Paper chromatogram were run on Whatman No.1 filter paper by descending development technique using any one of the following solvent systems (V/V).

A. n-Butanol : Pyridine : Water (10 : 3 : 3)
B. n-Butanol : Ethanol : Water (40 : 11 : 19)
C. n-Butanol : Acetic acid : Water (6 : 2 : 1)
D. Ethyl acetate : Acetic acid : Water (3 : 1 : 1)
E. Ethyl acetate : Pyridine : Water (10 : 4 : 3)

The irrigated papers were dried at room temperature and sugars were identified on chromatograms by dipping in, or spraying with any one of the following reagents.

(A) An aqueous saturated solution of silver nitrate (1 ml) diluted with acetone (500 ml); followed by 0.5 M sodium

hydroxide in ethanol. The chromatogram were washed with 2 % sodium thiosulphate solution followed by water.

(B) An alcoholic 1 % solution of aniline oxalate, followed by heating at 120^{0}C for 10 minutes.

(C) An alcoholic 0.1 % solution of p-anisidine and phthalic acid followed by heating at 100^{0}C for 10 minutes.

2.1.2.2 : VACUUM LIQUID CHROMATOGRAPHY (VLC) :

The concept of vacuum liquid chromatography (VLC) is a recent development in the field of chromatographic separation (Coll and Bowden, 1980). It is a column chromatography under reduced pressure and the column is packed with TLC grade silica gel. This new technique is applied in order to fractionate the crude extracts to obtain either a pure compound or mixtures containing less number of compounds.

In this technique a glass column of about 25 cm in length and 3-5 cm in diameter having a system fitted with a water pump and a collecting flask at the bottom is taken (Figure 2.1). Fine silica gel (mark 60 PF_{254}) is used as an adsorbent. The gel is packed into the column under an applied vacuum to give a bed of about 5 cm in height. The mixture to be separated is then preadsorbed in the required amount of the same gel (packed in the column) and is layered on the top of the bed or applied as a solution on the top of the bed. A gradient elution is then carried out with solvents of

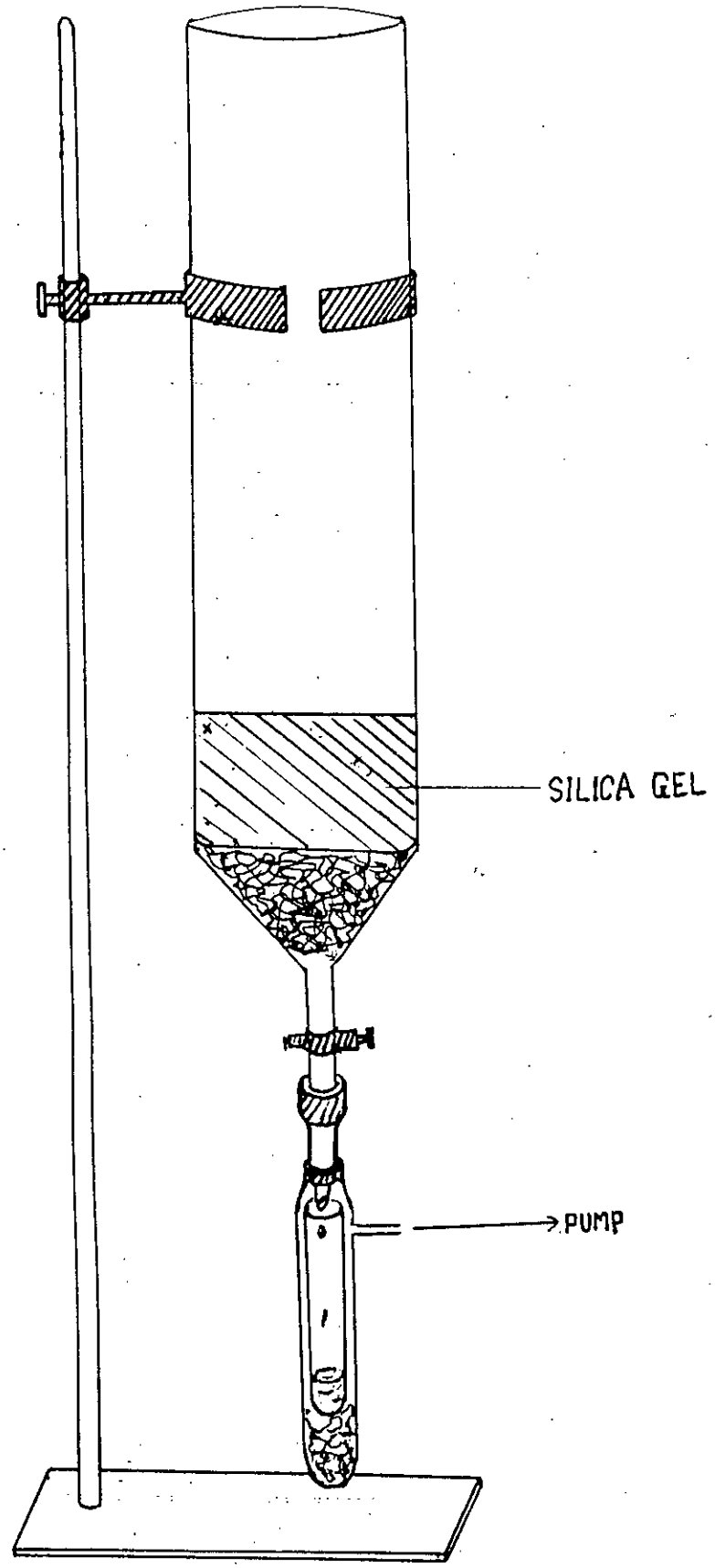


Fig. 2.1 Vacuum liquid chromatography (**VLC**)

increasing polarity until more polar components of the mixture are eluted. The effluents are collected manually in fractions of about 15 ml in test tubes and then the eluates from the tubes are monitored by TLC. The fractions giving identical spots in TLC are pooled together and are concentrated in a rota evaporator under reduced pressure.

The advantage of this technique in comparison to column chromatography is that it requires less amounts of eluting solvents, minimum quantity of solid adsorbents and a shorter time.

2.1.3.0 : THIN LAYER CHROMATOGRAPHY (TLC) :

2.1.3.1 : PREPARATION OF TLC PLATES :

Glass plates (20 X 20 cm ; 6 X 2 cm) are washed with detergent, water, distilled water and finally with acetone. Special care is taken in handling the tubes to avoid any contamination. Glass plates are then spreaded with a slurry of **silica gel 60 PF_{254}** in distilled water (1 : 2) to have a layer of ~0.50 mm in thickness to act as a stationary phase. After air drying, the plates are further dried at 110^0C in an oven for about an hour.

2.1.3.2 : SAMPLE APPLICATION (Spotting the plates) :

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

2.1.3.3 : PREPARATION OF TLC AND PTLC TANKS AND DEVELOPMENT OF CHROMATOGRAM :

Required amount of a suitable solvent system is poured into a chromatographic glass tank. The tank is then covered with a lid and kept for a certain period for attainment of saturation. A filter paper is usually introduced into the tank in order to promote the process of saturation. A spotted TLC plate is then placed in the saturated tank so that the solvent system applied at the bottom of the tank remains below the point of spot application in the plate. The chromatogram is then developed in an ascending manner. During development as the solvent rises upward, the plate becomes gradually moistened. Adequate care must be taken so that the solvent front does not travel beyond the upper end of the silica coated surface of the TLC plate. AS soon as the solvent front rises almost near the upper end of the silica coated plate, it is taken out and dried in an oven at 105^{0}C (**Donald et al., 1976**) for visualization by using various spray reagents or in an iodine chamber.

2.1.3.4 : PREPARATIVE THIN LAYER CHROMATOGRAPHY :

Fine silica gel (Mark 60 PF_{254}) is used to prepare PTLC plates. Usually glass plates (20 X 20 cm) are used for this purpose. At first the plates are cleaned and dried. A slurry is prepared by mixing silica gel (32 gm.) with distilled water (64 ml.). The slurry is then spreaded over the clean dry plates so that it makes a thickness of ~0.5 mm. . The plates so prepared is then allowed to set in air at room temperature for about an hour or two. These are then activated and made ready for use by heating

them at 110°C in an oven for about 2 hours. The activated plates are stored in a desiccator. The sample to be separated is dissolved in a very small amount of a suitable solvent and applied on the plates as a fine thin band near the baseline by using glass capillary tube. The plates are then developed in an appropriate solvent system predetermined by TLC. In some cases double or triple developments are performed in order to assure a maximum separation of the bands. The separated bands are visualized by the use of spray reagents and UV-light. Silica gel with the adsorbed substance is scrapped off with the help of a spatula and the adsorbed compound on silica gel is dissolved in a solvent of greater polarity than that used for developing the chromatogram. The silica gel is filtered off and the filtrate is concentrated in a rota evaporator to get back the separated pure substance.

2.1.3.5 : DETECTION OF COMPOUNDS ON THE DEVELOPED CHROMATOGRAM:

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

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

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

(C) Vanillin-sulphuric acid spray : The developed chromatoplates are sprayed with vanillin-sulphuric acid reagent and then heated at 110°C for 10 minutes. The resolved

compounds were identified with the development of specific colour (Mathews, 1963).

(D) Potassium permenganate spray : The chromatogram was sprayed with 0.5 % potassium permenganate reagent. The resolved compounds were identified with the development of colour instantly.

(E) DRAGENDROFF`S REAGENT : The presence of an alkaloid is detected by the appearance of an orange-red spot on spraying the developed chromatoplate with Dragendroff`s reagent.

2.1.3.6 : PREPARATION OF REAGENTS INCLUDING SPRAY REAGENTS FOR CHROMATOGRAM :

(A) **Vanillin sulphuric acid spray reagent** : Sulphuric acid (400 ml) and absolute alcohol (150 ml) is mixed in a beaker (kept in an ice-bath). Vanillin (0.25 gm) is added to this mixture of alcohol and sulphuric acid, cooled. Thus vanillin-sulphuric acid spray reagent is prepared.

(B) **Potassium permenganate spray reagent** : potassium permenganate (500 mg) is dissolved in distilled water (100 ml). Thus 0.5 % potassium permenganate spray reagent is prepared.

(C) **Ninhydrin spray reagent** : The standard reagent for identifying amino acids is ninhydrin (triketohydrindenehydrate). It is a 0.1 % solution of ninhydrin in acetone and is prepared by dissolving ninhydrin (100 mg) in acetone (100 ml).

(D) Dragendorff`s reagent :

Solution 1 : Bismuth subnitrate (600 mg) is dissolved concentrated Hydrochloric acid (2 ml) and water (10 ml).

Solution 2 : Potassium iodide (600 mg) is dissolved in water (10 ml).

Solution 1 and solution 2 are mixed with dilute HCL (22 ml = water 15 ml + Acid 7 ml) and the mixture is finally diluted with water (400 ml).

(D) Mayer`s reagent : Mercuric chloride ( 1.4 gm ) is dissolved in distilled water ( 60 ml ) and poured into a solution of potassium iodide ( 5 gm ) in water ( 10 ml ). Finally the volume of the solution is made 100 ml by adding required amount of water. This is Mayer`s reagent.

2.1.3.7 : THE R_f VALUE :

R_f value is defined as the ratio of the distance travel by a substance to the distance travel by the solvent (Figure 2.2).

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

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

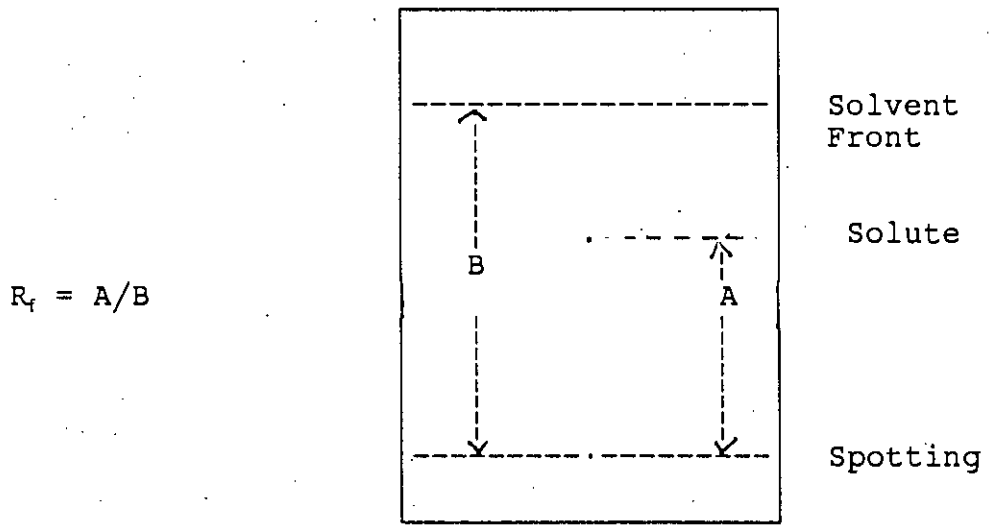


Fig. 2.2: A TLC plate for calculation of R_f value.

2.1.4.0 : GAS LIQUID CHROMATOGRAPHY (GLC) :

GLC for sugars was conducted with PYE UNICAM 4500U chromatograph fitted with flame ionization detector and either glass capillary column (3 % ov-225 2300 X 0.02 cm i.d.) or quartz capillary column (CP sil-88 1250 X 0.02 cm i.d.). The separations were performed at 160-220^{0}C, 3^{0}C min^{-1}, with hydrogen flow rate 45 ml min^{-1}. Peak areas were calculated with a LKB 3390A integrator.

GLC for fatty acids was conducted with a PYE UNICAM 4500U chromatograph fitted with flame ionization detector and quartz

capillary column (SGE 2500 X 0.02 cm i.d). The separations were performed at 90-115^{0}C, 3^{0}C min^{-1}, with nitrogen flow rate of 30 ml min^{-1}. Peak areas were calculated with a LKB 2220A integrator.

2.1.5.0 : SPECTRAL INVESTIGATIONS :

2.1.5.1 : INFRARED SPECTROSCOPY (IR) :

The infrared spectra of all the samples were recorded in a Simatzu IR-470 spectrometer (in KBr pellet/nujol).

2.1.5.2 : NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPY :

(A) ^{1}H-NMR and ^{13}C-NMR spectra were recorded in deuterated chloroform $CDCL_3$, pyridine and benzene using tetramethylsilane (TMS) as an internal standard on a Bruker WH-500 MHz spectrometer.

(B) 2-D NMR were recorded in benzene

2.1.6.0 : ANALYSIS OF FATTY ACIDS :

For analysis of the fatty acids the pet ether or chloroform extract of the plant material is saponified and the individual fatty acids thus obtained is analyzed as such by High Performance Liquid chromatography (HPLC) or as their methyl ester derivative by GLC.

2.1.6.1 : SAPONIFICATION OF PET ETHER/CHLOROFORM EXTRACT AND PREPARATION OF METHYL ESTER DERIVATIVES OF FREE FATTY ACIDS :

(A) Saponification of pet ether/chloroform extract :

A portion of the dried pet ether/chloroform extract (500 mg) is taken in a pear-shaped flask. Methanolic sodium hydroxide (0.5M, 10 ml) is added to it and shaken well. It is refluxed in a boiling water bath for 30 minutes. The mixture is then evaporated to dryness by rotary evaporation. Water (25 ml) is added to the mixture and the solution is transferred into a seperatory funnel. The non-saponified materials were separated from the saponified portion (aqueous layer) by extraction with hexane. The aqueous layer containing the fatty acids as Na-salt is acidified with ortho-phosphoric acid and the pH of the solution is adjusted to 2.5. The free fatty acids were then extracted with n-hexane (3 X 10 ml). To the hexane extract suitable amount of anhydrous sodium/magnesium sulphate is added and kept overnight to remove traces of water if any. It is filtered in a pear-shaped flask and evaporated to dryness which contains the saponified free fatty acids.

(B) Preparation of methyl ester derivatives of free fatty acids :

Boron-trifluoride methanol (BF_3-MeOH) complex (2ml) is added to the dry fatty acids and refluxed for about 6-10 minutes in

a boiling water bath. The mixture is evaporated to dryness in a rota evaporator and water (10 ml) is added to it. The whole content is transferred into a small separatory funnel and extracted with n-hexane (3 X 25 ml). The aqueous layer is discarded. The hexane extract is made free from water by treating it with anhydrous sodium sulphate. It is filtered. The filtrate is treated with decolourising charcoal and filtered again. The clean filtrate is concentrated to a very small volume and is analyzed by GLC.

2.1.7.0 : ACETYLATION OF FREE HYDROXYL GROUPS OF SUGAR (Ref) :

Method A : The dry substance (100 mg), anhydrous powdered sodium acetate (50 mg) and acetic anhydride (4 ml) is taken in a 25 ml ground joined round bottom flask fitted with a condenser. It is refluxed in a steam bath for about 2 hours. The content is poured into ice-water (4 ml) with stirring. The crystals formed is filtered and washed with cold distilled water. The product obtained is recrystallized from rectified spirit to get the pure acetyl derivative.

Method B : In a 25 ml round bottom flask fitted with a small condenser the dry substance (100 mg) is taken. Dry pyridine (1 ml) is added to the substance with shaking and then acetic anhydride (0.4 ml) is added to it. After the initial reaction has subsided, the reaction mixture is refluxed for 5 minutes in a water

bath. The reaction mixture is cooled and poured into ice-water (4 ml). The crystals are filtered and washed with 1M cold hydrochloric acid (10 ml X 3) and finally with cold distilled water. The product so obtained is recrystallized from rectified spirit.

2.1.8.0 : TESTS FOR SUGARS AND STEROIDS :

(A) Phenol-sulphuric acid test for sugar (Dubous et al., 1956) :

Sample solution (0.5 ml) is taken in a test tube (100 cm long) in such a way that it does not touch the sides of the test tube. Phenol solution (5 % , w/v, 0.5 ml) is added from a dispenser to the sample solution taking sufficient care not to touch the walls of the tube. Sulphuric acid (98 %, 2.5 ml) is added directly and quickly into the sample using a dispenser. Sufficient care is taken to avoid splashing of any acid out of the tube. Development of a reddish brown colour confirms the presence of sugar in the sample.

(B) Salkowski test for steroids (Finar, 1982) :

The extracted substance (2 mg) is taken in a test tube containing chloroform (2ml) alternatively chloroform extract (2 ml) of the sample is taken in a test tube. Concentrated sulphuric acid (1 ml) is slowly added from the side of the test tube.

Development of a red colour in the chloroform layer indicates the presence of a steroid.

(C) **Liebermann-Burchard`s test for steroids (Finar, 1982) :**

The extracted substance (2 mg) is taken in a test tube containing chloroform (2 ml). Acetic anhydride (2 ml) is added to it followed by addition of concentrated sulphuric acid (1 ml). Development of a greenish colour which turns blue on standing indicates the presence of a steroid in the sample concern.

3.0 CHAPTER 3

3.1.0 : EXPERIMENTAL :

3.1.1.0 : CHEMICAL INVESTIGATION OF *LEUCAS ZEYLANICA LINN.* AND *GREWIA MICROCOS LINN.*

3.1.1.1 : MATERIALS :

Under the current project two materials were used for Chemical investigation. These were the stems of the herb *Leucas Zeylanica Linn.* and the stems of the shrub *Grewia microcos Linn.* *Leucas Zeylanica Linn.* was collected from Dinajpur district during the month of August, 1997 while *Grewia microcos Linn.* was collected from the bushy area of the Jahangir Nagar University campus, Savar, Dhaka district during the month of April, 1998. These were correctly identified as the *Leucas Zeylanica Linn.* and the *Grewia microcos Linn.* by comparing them with their voucher specimens that have been lodged at the Herbarium in the Botany Department of Dhaka University, Bangladesh.

The stems and leaves of the two plants were separated manually into two parts. The stems were then dried in the air. The dry stems of each specimen were separately cut into small pieces (~ 0.5 cm.) and made ready for soxhlet extraction by grinding them into fine powders in a cyclotech grinding machine.

3.1.2.0 : SOXHLET EXTRACTION OF *LEUCAS ZEYLANICA LINN.* WITH PET ETHER, EtOAC, MeOH AND 80% EtOH :

The powdered stem of *Leucas Zeylanica Linn.*(750 gm.) was extracted exhaustively in a soxhlet apparatus successively with organic solvents of increasing polarity e.g. pet ether (60-80^{0}C), EtOAC and MeOH and 80 % EtOH. The extracts were filtered and concentrated to small volumes under reduced pressure by rotary evaporation. These were called Pet ether extract (A), EtOAC extract (B) and MeOH (C) and 80% EtOH extract (W).

3.1.2.1 : FRACTIONATION OF PET ETHER EXTRACT (A) BY VLC :

The concentrated pet ether extract (A) was subjected to VLC over TLC grade silica gel G_{250}. The column was eluted first with pet ether (60-80^{0}C, total 300 ml), then with pet ether : EtOAC (90 : 10 ; 80 : 20 ; 70:30 ; 60 : 40 ; 50 : 50 ; 40 : 60 ; 30 : 70 ; 20 : 80 ; 10 : 90 , 0 : 100, total 1000 ml) and finally with EtOAC : MeOH (90 : 10 ; 80 : 20 ; 70:30 ; 60 : 40 ; 50 : 50 ; 40 : 60 ; 30 : 70 ; 20 : 80 ; 10 : 90 , 0 : 100, total 1000 ml). Eluates were collected in test tubes in about 15 ml portion. Eluate from each tube was monitored by TLC and the eluates from tubes showing similar TLC behaviour were pooled together in different fractions. Six such fractions were collected. These were named as fraction LZP_1 , LZP_2 , LZP_3 , LZP_4 , LZP_5 and LZP_6 .

3.1.2.2 : ISOLATION OF A MIXTURE OF FATTY ACID ESTERS LZFA FROM THE VLC SEPARATED FRACTIONS LZP_1 , LZP_2 AND LZP_3 :

The fractions LZP_1 , LZP_2 and LZP_3 were concentrated and subjected to TLC in a solvent system of pet ether and EtOAC in different ratios and the TLC`s were visualized by using different spray ragents and in iodine chamber. In all the TLC`s a number of very closely related spots were observed. It was difficult to separate them into individual components by CC and PTLC. The fractions when tried to be dried in a vacuum desiccator they fall into creamy masses. These were mixed together (~ 1000 mg) and designated as LZFA.

3.1.2.3 : SAPONIFICATION OF LZFA AND PREPARATION OF ESTER DERIVATIVES OF FREE FATTY ACIDS :

The fraction LZFA was saponfied and the ester derivatives of the saponified free fatty acids were prepared by the method as described in the section 2 (2.1.6.1).

3.1.2.4 : ISOLATION OF PURE COMPOUNDS FROM THE VLC SEPARATED FRACTIONS LZP_4 , LZP_5 , LZP_6 OF PET ETHER EXTRACT :

The fraction LZP_4 on concentration (30 ml) and standing undisturbed at room temperature for about 10 days yielded a white needle like crystal. The mother liquior was decanted and the

crystals were thorougly washed first with n-hexane and then with pet-ether. These were then dried in a vacuum desiccator and subjected to TLC in a solvent system of Pet ether : EtOAC (2 : 1) showing a single spot when visualized by various sparaying reagents and also in iodine vapour. The mother liquior on standing gave two other such crystalline crops having similar TLC behaviour with the first one. The three crops were mixed together giving the pure compound LZ1 (100 mg). The remainder of mother liquer and washings on concentration gave a very minor amount of a contaminated product and hence discarded.

No individual compound in analyzable amount could be isolated from the fractions LZP_5 and LZP_6 .

3.1.2.5 : FRACTIONATION OF EtOAC EXTRACT (B) BY VLC :

The concentrated EtOAC (B) extract was subjected to VLC over TLC grade silica gel G_{250}. The column was eluted first with pet ether (60-80^{0}C, total 300 ml), then pet ether : EtOAC (90 : 10 ; 80 : 20 ; 70:30 ; 60 : 40 ; 50 : 50 ; 40 : 60 ; 30 : 70 ; 20 : 80 ; 10 : 90 , 0 : 100, total 1000 ml), and finally with EtOAC and MeOH (90 : 10 ; 80 : 20 ; 70:30 ; 60 : 40 ; 50 : 50 ; 40 : 60 ; 30 : 70 ; 20 : 80 ; 10 : 90 , 0 : 100, total 1000 ml). Eluates were collected in test tubes in about 15 ml portion. Eluate from each test tube was monitored by TLC and the eluates from the tubes having similar TLC behaviour were pooled together in different

fractions. Five such fractions could be collected. These were named as fraction LZE_1 , LZE_2 , LZE_3 , LZE_4 and LZE_5.

3.1.2.6 : ISOLATION OF PURE COMPOUNDS FROM VLC FRACTIONATED FRACTIONS LZE_1 , LZE_2 , LZE_3 , LZE_4 AND LZE_5 OF EtOAC EXTRACT :

When the fractions LZE_1 and LZE_2 were subjected to TLC under different solvent systems and visualized by different spray reagents and also in iodine chamber continious bands were raised from the point of sample application upto the solvent front. Hence further studies on these fractions were not continued.

TLC examination of the fraction LZE_3 in EtOAC : pet ether (3 : 1) exhibited the presence of three spots. The fraction was subjected to CC (60 X 3 cm) using silical gel as stationary phase. The column was eluted first with pet ether (60-80^{0}C, total 300 ml), then with pet ether : EtOAC (90 : 10 ; 80 : 20 ; 70:30 ; 60 : 40 ; 50 : 50 ; 40 : 60 ; 30 : 70 ; 20 : 80 ; 10 : 90 , 0 : 100, total 1000 ml), and finally with EtOAC : MeOH (90 : 10 ; 80 : 20 ; 70:30 ; 60 : 40 ; 50 : 50 ; 40 : 60 ; 30 : 70 ; 20 : 80 ; 10 : 90 , 0 : 100, total 1000 ml). Eluates were collected in test tubes in about 15 ml. portion. Eluate from each test tube was monitored by TLC and the eluates from the tubes showing similar TLC behaviour were pooled together in different fractions. Three such fractions could be collected. From two of these fractions

compounds could not be isolated in analyzable amounts. The third fraction on concentration when subjected to TLC in EtOAC: Pet ether (3 : 1) gave a single spot in TLC with little contamination. The fraction was further purified by applying the technique of PTLC as described in the section 2 (2.1.3.4) to give a pure ash coloured amorphous compound LZ2 (35 mg.)

As the fraction LZE_4 was subjected to TLC in a solvent system EtOAC : Pet ether (5 : 1) one bright spot with tailings was observed. The fraction was concentrated and purified by repeated PTLC using solvent system EtOAC : Pet ether (5 : 1). A single spotted brown amorphous substance LZ3 (30 mg) was obtained.

When the fraction LZE_5 was subjected to TLC using 100% EtOAC as solvent it gave a single spot but with tailings above and below the spot. The fraction was treated with a suitable amount of decolourising charcoal for about two hours, warmed and filterd. The filtrate on concentration and standing at desk temperature for several days yielded a white crystalline substance. The mother liquor was decanted. The substance was washed with EtOAC while a portion of the crystals went into solution. The washed crystals were dried in a vacuum desiccator. The washings from the crystals were added to the mother liquior, concentrated and allowed to stand at room temperature again to yield another crop of the same substance which was washed with EtOAC and dried in a vacuum desiccator. The two crops when subjected to TLC with 100 % EtOAC as

mobile phase and visualized by using different pray reagents similar TLC behaviour of a single spotted compound could be observed. Hence the two fractions were mixed together to give the substance LZ4 (20 mg). The remainder of the mother liquior and the washings together on concentration still afforded a very minor amount of a contaminated product and was discarded.

3.1.2.7 : PREPARATION OF ACETYL DERVATIVE LZA2 FROM THE COMPOUND LZ2 :

LZ2 (15 mg) was acetylated by following the procedure 2.1.7.0 (**Method B**) as described in section 2 yielding the acylated product LZA_2 (21 mg).

3.1.2.8 : ISOLATION OF COMPOUNDS FROM MeOH EXTRACT (C) :

The concentrated MeOH extract (C) on standing at room temperature for about 10 days yielded a needle shaped crystalline substance. The mother liquior was decanted and the crystals were washed several times with dry methanol. Chlorform (30 ml) was then added to these crystals and left overnight. Chloroform was decanted and the crystals were dried in a vacuum desiccator to give the Compound LZ5 (1000 mg).

The mother liquior and washings from the compound LZ_5 was concentrated and subjected to VLC over TLC grade silica gel G_{250}. The column was eluted first with pet ether (60-80^{0}C, total 300 ml), then with pet ether : EtOAC (90 : 10 ; 80 : 20 ; 70:30 ; 60 : 40 ; 50 : 50 ; 40 : 60 ; 30 : 70 ; 20 : 80 ; 10 : 90 , 0 : 100, total 1000 ml), and finally with EtOAC and MeOH (90 : 10 ; 80 : 20 ; 70:30 ; 60 : 40 ; 50 : 50 ; 40 : 60 ; 30 : 70 ; 20 : 80 ; 10

: 90 , 0 : 100, total 1000 ml). Eluates were collected in test tubes in about 15 ml. portion. Eluate from each test tube was monitored by TLC and the eluates from the tubes having similar TLC behaviour were pooeld together in different fractions. Eight such fractions could be collected. These were named as fractions LZM_1 , LZM_2 , LZM_3 , LZM_4 , LZM_5 , LZM_6 , LZM_7 AND LZM_8. But no organic compound of suitable amount could be isolated from these fractions by applying all possible isolation techniques.

3.1.2.9 : ISOLATION OF A WATER SOLUBLE FRACTION LZW FROM 80 % EtOH EXTRACT :

The 80 % EtOH extract obtained from soxhlet extraction was concentrated, filtered and evaporated to dryness by rotary evaporation. It was dissolved in distilled water, filtered and filtrate is evaporated dryness by rotary evaporation. The process of dissolution and evaporation was continued till no precipitate or any suspension is obtained on addition of distilled water to the evaporated mass. The clean solution was then dried by rotary evaporation and finally in a vacuum desiccator to give the fraction LZW (10 mg).

3.1.3.0 : SOXHLET EXTRACTION OF *GREWIA MICROCOS LINN.* WITH PET ETHER, EtOAC, AND MEOH :

The powdered stem of *Grewia microcos Linn.*(900 gm.) was

extracted exhaustively in a soxhlet apparatus successively with organic solvents of increasing polarity e.g. pet ether (60-80^{0}C), EtOAC , MeOH and 80 % EtOH. The extracts were filtered individually and concentrated to small volume under reduced pressure by rotary evaporation. These were called Pet ether extract (D), EtOAC extract (E) and MeOH extract (F).

3.1.3.1 : ISOLATION OF COMPOUNDS FROM PET ETHER EXTRACT (D) :

The pet ether extract on concentration and standing undisturbed at room temperature for several days gave a white crystalline product. The mother liquor was decanted. The crystalline substance was washed first with n-hexane and then with pet ether. The washed crystals were dried in a vacuum desiccator. The washings from the crystals were added to the mother liquior, concentrated and allowed to stand at room temperature yielding another crop of the white substance which was washed with n-hexane and pet ether. It was dried in a vacuum desiccator. The two crops when subjected to TLC with pet ether : EtOAC (3:1) revealed similar TLC behaviour of a single substance and hence were mixed together. This was given the name GMP1 (150 mg). The remainder of the mother liquior and washings together was concentrated and subjected to further investigation.

3.1.3.2 : ISOLATION OF THE FRACTION GMFA FROM PET ETHER EXTRACT AFTER SEPARATION OF THE COMPOUND GMP1 :

The remainder of the mother liquior and the washings together after separation of the compound GM_1 was concentrated to give a oily liquid (~ 30 ml). This was left undisturbed at room

temperature. On standing for several days it gave a semicrystalline mass. The mother liquior was decanted and the mass was washed very quickly with n-hexane and dissolved in chloroform. on standing for several days on the chloroform was naturally evaporated leaving a crysatlline mass. This on drying in a vacuum desiccator gave the fraction GMFA (200 mg). GMFA gave single spot in TLC (Solvent system = pet ether : chloroform = 1 : 2, R_f value = 0.78, visualized in iodine vapour as a brown spot).

3.1.3.3 : FRACTIONATION OF EtOAC EXTRACT (E) BY VLC :

The concentrated EtOAC extract (E) was subjected to VLC over TLC grade silica gel G_{250}. The column was eluted first with pet ether (60-80^{0}C, total 300 ml), then with pet ether : EtOAC (90 : 10 ; 80 : 20 ; 70:30 ; 60 : 40 ; 50 : 50 ; 40 : 60 ; 30 : 70 ; 20 : 80 ; 10 : 90 , 0 : 100, total 1000 ml), and finally with EtOAC : MeOH (90 : 10 ; 80 : 20 ; 70:30 ; 60 : 40 ; 50 : 50 ; 40 : 60 ; 30 : 70 ; 20 : 80 ; 10 : 90 , 0 : 100, total 1000 ml). Eluates were collected in test tubes in about 15 ml portion. Eluate from each test tube was monitored by TLC and the eluates from the tubes showing similar TLC behaviour were pooeld together in different fractions. Seven such fractions were collected. These were named as fraction GME_1 , GME_2 , GME_3 , GME_4, GME_5, GME_6 and GME_7.

3.1.3.4 : ISOLATION OF THE COMPOUND GME2 FROM VLC FRACTIONATED FRACTION GME_4 OF EtOAC EXTRACT :

The VLC seperated fraction GME4 on concentration and standing undisturbed at room temperature yielded a semisolid mass. When treated with methanol and chloroform (9 : 1) most of this mass

went into solution. It was filtered to remove the minor amount of insoluble material. TLC of the filtrate in MeOH : EtOAC (1:1) when visualized in iodine vapour and vanillin-sulphuric acid spray followed by heating at 110^{0}C gave a single spot with tailings above and below the spot. The filtrate was then concentrated and subjected to PTLC for twice using MeOH and EtOAC as solvent in the ratio 1 : 1 to get a single spotted compound GME2 (25 mg).

Since TLC behaviour of the other VLC fractionated fractions were not viable for seperation of individual compounds further studies on these fractions were not continued.

3.1.3.5 : FRACTIONATION OF MeOH EXTRACT (F) BY VLC :

The concentrated MeOH extract (F) was subjected to VLC over TLC grade silica gel G_{250}. The column was eluted first with pet ether (60-80^{0}C, total 300 ml), then with pet ether : EtOAC (90 : 10 ; 80 : 20 ; 70:30 ; 60 : 40 ; 50 : 50 ; 40 : 60 ; 30 : 70 ; 20 : 80 ; 10 : 90 , 0 : 100, total 1000 ml), then with EtOAC : MeOH (90 : 10 ; 80 : 20 ; 70 : 30 ; 60 : 40 ; 50 : 50 ; 40 : 60 ; 30 : 70 ; 20 : 80 ; 10 : 90 , 0 : 100, total 1000 ml) and finally with MeOH : Water (90 : 10 ; 80 : 20 ; 70 : 30 ; 60 : 40 ; 50 : 50 ; 40 : 60 ; 30 : 70 ; 20 : 80 ; 10 : 90 , 0 : 100, total 1000 ml.) Eluates were collected in test tubes in about 15 ml. portion. Eluate from each test tube was monitored by TLC and the eluates from the tubes showing similar TLC behaviour were pooeld

together in different fractions. Nine such fractions were collected. These were named as fraction GMM_1 , GMM_2 , GMM_3 , GMM_4 GMM_5, GMM_6, GMM_7 GMM_8 and GMM_9.

3.1.3.6 : ISOLATION OF THE COMPOUNDS GMM3 FROM VLC SEPERATED FRACTIONS GMM_7 AND GMM_8 OF MeOH EXTRACT :

The fractions GMM_7 AND GMM_8 on concentration and standing at room temerature yielded semisolid masses. The masses obtained on decantation of the mother liquiors were found to be insoluble in Chloroform, EtOAC and MeOH. But these were readily soluble in water with a very minor amount of suspended particles. The suspended particles were removed by filtration. Both these fractions on concentration gave positive test for alkaloid on treatment with Dragendorff`s reagent. The two fractions were mixed together, evaporated to dryness by rotary evaporation. The mass was redissolved in distilled water, filtered to remove any suspended impurity, evaporated to dryness in a rota evaporator and finally dried in a vacuum desiccator to give the compound GMM3 (50 mg).

The VLC seperated other concentrated fractions of MeOH extract (F) when subjected to TLC using different solvent systems in silica coated TLC plates and the developed chromatoplates were visualized by application of different spray reagents, the responds obtained were not in favour of isolating single component compounds from them. As a result further studies are not pursued on them.

3.1.4.0 : PROPERITIES OF THE PURE COMPOUNDS OR FRACTIONS ISOLATED FROM *LEUCAS ZEYLANICA* LINN. AND *GREWIA MICROCOS LINN.*

3.1.4.1 : PROPERTIES OF THE ISOLATED FRACTION LZFA.

Physical appearance : A crsytalline creamy mass

Solubility : Soluble in pet.ether, n-hexane and chloroform.

IR Spectrum ν_{max}^{KBr} cm^{-1} : 2900, 2850, 1720, 1440, 1370, 1255, 1080, 1020, 800

Retention times of the BF3-MeOH esterified derivatives of the saponified fraction LZFA :

1.09, 3.52, 6.89, 11.56, 14.38, 14.54, 15.00, 15.36, 16.02, 16.61 (major, palmitic acid), 20.78 (major, stearic acid), 20.61 (major, not found in the standard), 21.39 (major, Oleic acid) , 23.81, 23.71, 25.88, 26.38, 29.31, 31.12 , 36.44

Retenion times of the standard :

nonanoic acid (1.08), capric acid (1.31), undecanoic acid (3.51), caprylic acid (4.96), lauric acid (6.86), myristic acid (11.55), palmitic acid (16.65), stearic acid (20.76) oleic acid (21.45), arachidic acid (24.84), behenic acid (31.23).

3.1.4.2 : PROPERTIES OF THE ISOLATED COMPOUND LZ1 :

Physical appearance : White needle shape crystal

Solubility : Highly soluble in pet. ether

Melting point : 157-158^{0}C

R_f value : 0.73 (over silica gel, Pet ether : EtOAC, 2 : 1 as the mobile phase).

IR Spectrum ν_{max}^{KBr} cm^{-1} : 3420 hydroxyl group (-OH)
2900, 2850 ali. C-H str.
1460 C-H bending for ($-CH_2-$) group
1375 C-H bending for ($-CH_3$) group
1060 C-O str. for 1^0 (-OH) group
960, 798, 765.

^1H NMR (500 MHz, $CDCl_3$, δ PPM) : 0.657 (3H, d,21-Me); 0.820 (3H, d, 26-Me); 0.768 (3H, d, 27-Me) ; 1.033 (3H,CH3); 3.476 (1H, dq, H-3); 4.972 (1H, dd, H-23) ; 5.106 (1H, dd, H-22) 5.323 (1H, br.s, H-7) 1.500 (m) ; 1.932 (m), 2.182 (m).

EIMS spectrum (500 MHz, $CDCl_3$) : m/e 412 [$C_{29}H_{48}O$]$^{+\cdot}$; 300 [C22H37]+ ; 273 [C19H29O]+ ; 255 [C19H27]+ ; 271 [C18H27O]+ ; 159 [C12H15]+ ; 55 [C4H7]+

2D NMR spectrum : Is given in results and discussion (chapter 4)

3.1.4.3 : PROPERTIES OF THE ISOLATED COMPOUND LZ2 :

Physical appearance : Ash color amorphous substance

Solubility : Soluble in MeOH (hot)

Melting point : 294-295^{0}C

R_f value : 0.26 (over silica-gel, EtOAC : Pet ether, 3:1 as the mobile phase).

IR Spectrum ν_{max}^{KBr} cm^{-1} : 3400 hydroxyl group (-OH)
2910,2880 ali. C-H str.
1450 C-H bending for($-CH_2-$) group
1370 C-H bending for ($-CH_3$) group
1250, 1050 C-O- str.
970, 960 C-H bending
840,800.

^{1}H NMR (500 MHz, pyridine, δ PPM) :

0.815	(3H, d, Me-27)
0.868	(6H, s, C18-19)
0.911	(3H, d, Me-26)
1.001	(3H, d, Me-21)
1.800	(2H, d, H-6)
2.383	(1H, t, H-4a)
2.649	(1H,d, H-4b)
3.889	(2H, d, H-3',)
3.992	(1H, s, H-4' + 6b')
4.220	(2H, s, H-6a')
4.337	(1H, d, H-5')
4.487	(1H, s, H-3')
4.982	(2H, d, H-23, 1')
5.126	(1H, dd, H-22)
5.277	(1H, s, H-7)

^{13}C NMR (deuteropyridine, δ PPM) : C1-36.5 ; C2-28.57 ; C3-75.38 ; C4-32.19; C5-56.96 ; C6-30.30 ; C7-129.51 ; C8-138.85 ; C9-56.11 ; C10-50.39 ; C11-39.86 ; C12-46.09 ; C13-56.29, C14-51.45 ; C15-29.33 ; C16-40.80 C17-29.51 ; C18-36.42 ; C19-37.52 ; C20-32.21 ; C21-39.38 ; C22-121.93 ; C23-135.81 ; C24-62.89 ; C25-56.87 ; C26-19.89 ; C27-39.99 ; C28-150.22 ; C29-123.93 ; C1'-102.61 ; C2'-78.64 ; C3'-78.51 ; C4'-71.75 ; C5'-78.14 ; C6'-34.25

3.1.4.4 : PROPERTIES OF ACETYL DERIVATIVE OF LZ2 :

Physical state	:	Light yellow crystalline substance
solubility	:	Highly soluble in chloroform
R_f Value	:	0.77 (over silica gel, EtOAC : Chloroform = 1:1)
Amount	:	21 mg
IR Spectrum KBr ν_{max} cm^{-1}	:	3400, 2950, 2880, 2350, 1740, 1630, 1450, 1360, 1225, 1170, 1040, 910, 840, 800, 690, 600, 530
^{1}H NMR (60 MHz, $CDCl_3$ δ PPM)	:	0.50-1.70, 2.00, 4.00, 5.20

3.1.4.5 : PROPERTIES OF THE ISOLATED COMPOUND LZ_3 :

Physical appearance	: Amorphous brown coloured substance
Solubility	: Soluble in MeOH (hot)
Melting point	: 274^0 C
R_f value	: 0.65 (over silica-gel, EtOAC : Pet ether, 5:1 as the mobile phase).
IR Spectrum ν_{max}^{KBr} cm^{-1}	: 3380 hydroxyl group (-OH) 2900,2850,1610,1500 (Aromatic ring) 1450 C-H bending for ($-CH_2-$) group 1365 C-H bending for ($-CH_3$) group 1160, 1100, 1065, 1020 C-O- str. 800, 720 C-H bending
^1H NMR (500 MHz, pyridine, δ PPM)	: 0.785 (6H, s, C-18,19) 0.800 (3H, d, Me-29) 0.815 (3H, d, Me-27) 0.911 (3H, d, Me-26) 1.001 (3H, d, Me-21) 2.383 (1H, t, H-4a) 2.500 (m) 2.649 (1H,d, H-4b) 3.750 (m) 3.889 (2H, d, H-3',4') 3.992 (1H, s, H-3) 4.220 (2H, s, H-6') 4.337 (1H, d, H-1') 4.487 (1H, d, H-5') 4.982 (2H, d, H-23) 5.126 (1H, dd, H-22) 5.277 (1H, s, H-7)

3.1.4.6 : PROPERTIES OF THE ISOLATED COMPOUND LZ_4 :

Physical appearance	: White crystalline (round)
Solubility	: Soluble in MeOH (hot)
Melting point	: Above 300^0C (with decomposition)
R_f value	: 0.76 (over silica-gel, 100 % EtOAC as mobile phase).

IR Spectrum ν_{max}^{KBr} cm^{-1} : 3400 (-OH) 2900, 2850, 1720 1530, 1450, 1080 (broad) 800, 720 C-H bending 640, 600

^{1}H NMR (500 MHz, Pyridine, δ PPM) :

0.770	(1H,br. s, Me-7)
1.188	(1H, br.s, Me-8)
2.136	(1H, m, H-2)
2.190	(1H, dd,H-3)
2.410	(1H, s,2-OH)
4.221	(1H, m, H-6a')
4.272	(1H, m, H-3')
4.344	(1H, m,H-5')
4.434	(1H, m, H-4')
4.546	(1H, m, H-2')
4.874	(1H, br.s,OH proton)
5.042	(1H, br.s,H-6b')
5.435	(1H, dd, H-1')

^{13}C NMR (deuteropyridine, δ PPM) : C1-163.07 ; C2-29.94 ; C3-32.67 ; C4-26.66 ; C5-130.70 ; C6-123.06 ; C7-49.47 ; C8-39.26 ; C9-25.17 ; C10-149.44 ; 7-Me (17.12) ; 8-Me (14.27), C1'-109.94 ; C2'-87.64 ; C3'-76.82 ; C4'-72.48 ; C5'-75.55 ; C6'-62.07

3.1.4.7 : PROPERTIES OF THE ISOLATED COMPOUND LZ5 :

Physical appearance : Silky white needle crystalline substance

Solubility : Insoluble in chloroform, EtOAC and MeOH but readily soluble in water.

Melting point : 353-355^{0}C

Amount : 1000 mg

IR Spectrum ν_{max}^{KBr} cm^{-1} : 3450, 2900, 2850, 2350, 1380, 820

^{1}H NMR (500 MHz, Pyridine, δ PPM) : No signal

^{13}C NMR (deuteroPyridine, δ PPM) : No signal

3.1.4.8 : PROPERTIES OF THE ISOLATED FRACTION LZW :

Physical appearance : White mass (remains dry in a vacuum desiccator but absorbs moisture from the open atmosphere)

Solubility : Readily soluble in water

3.1.5.1 : PROPERTIES OF THE ISOLATED COMPOUND GMP1 :

Physical state : White crystalline substance

Solubility : soluble in pet ether and highly soluble in chloroform

R_f value : 0.75 (over silica gel, pet ether : EtOAC = 3 : 1)

Melting point : 267°C

Amount : 90 mg

IR Spectrum ν_{max}^{KBr} cm^{-1} : 3460, 2900, 2860, 1700, 1620, 1450, 1380, 1310, 1178, 1120, 1070, 1050, 1020, 1000, 980, 920, 790

3.1.5.2 : PROPERTIES OF THE ISOLATED COMPOUND GMFA :

Physical state : Semicrystalline mass

Solubility : Soluble in pet ether, chloroform, n-hexane

R_f value : 0.78 (over silica gel, pet ether : chloroform = 1: 2)

Melting point : 71°C

Amount : 200 mg

IR Spectrum ν_{max}^{KBr} cm^{-1} : 3420, 2900, 2850, 1720, 1500, 1480, 1370, 1260

3.1.5.3 : PROPERTIES OF THE ISOLATED COMPOUND GME2 :

Physical state : Light brown amorphous substance

Solubility : In a mixture of hot chloroform and MeOH

Melting point : 297^{0}C

R_f value : 0.82 (Over silica gel, EtOAC : MeOH = 1 : 1)

Amount : 25 mg.

IR Spectrum ν_{max}^{KBr} cm^{-1} : 3400, 2900, 2880, 1740 (boroad), 1620, 1540, 1450, 1430, 1360, 1220, 1250, 1160, 1100, 1070, 1010, 800, 610, 515

3.1.5.4 : PROPERTIES OF THE ISOLATED COMPOUND GMM3 :

Physical state : Amorphous, Light yellowish brown coloured

Solublity : Insoluble in pet ether, chloroform and methanol but readily soluble in water

Melting point : Decomposes above 350^{0}C

Amount : 50 mg

IR Spectrum ν_{max}^{KBr} cm^{-1} : 3400, 2900, 2880, 1700, 1620, 1440, 1380, 1175, 1050, 1000

4.0 CHAPTER 4

4.1.0: RESULTS AND DISCUSSION

4.1.1.0 : CHARACTERIZATION OF LZFA AS A MIXTURE OF FATTY ACID ESTERS :

The VLC separated fractions- LZP1, LZP2 and LZP3 of pet ether extract of *Leucas Zeylanica Linn.* were combined together because of their similar TLC behaviour, concentrated and left overnight to yield a crude creamy solid LZFA(250 mg). When it was subjected to TLC studies (solvent system of pet ether : EtOAC of different ratios) and each of the TLC plates was first exposed to iodine vapour in a closed glass chamber, a continuous band of yellowish colour was visualized. After removal of iodine stain on drying in air, each of the plate was treated with spray reagents like dilute $KMnO_4$ solution , Vanillin -sulphuric acid, a continuous band of pinkish colour was observed. Because of their very close R_f values, these could not be separated into individual components in spite of our repeated attempts employing usual chromatographic techniques. The bands in the IR spectrum at (Figure 4.1) at V 1720 cm-1, 1255 cm-1, 1080 cm-1 and 1020 cm-1 indicated that the solid creamy mass LZFA was the type of fatty acid esters (Harborne, 1973).

A portion of this solid mass was then saponified with methanolic sodium hydroxide and the free fatty acids obtained were

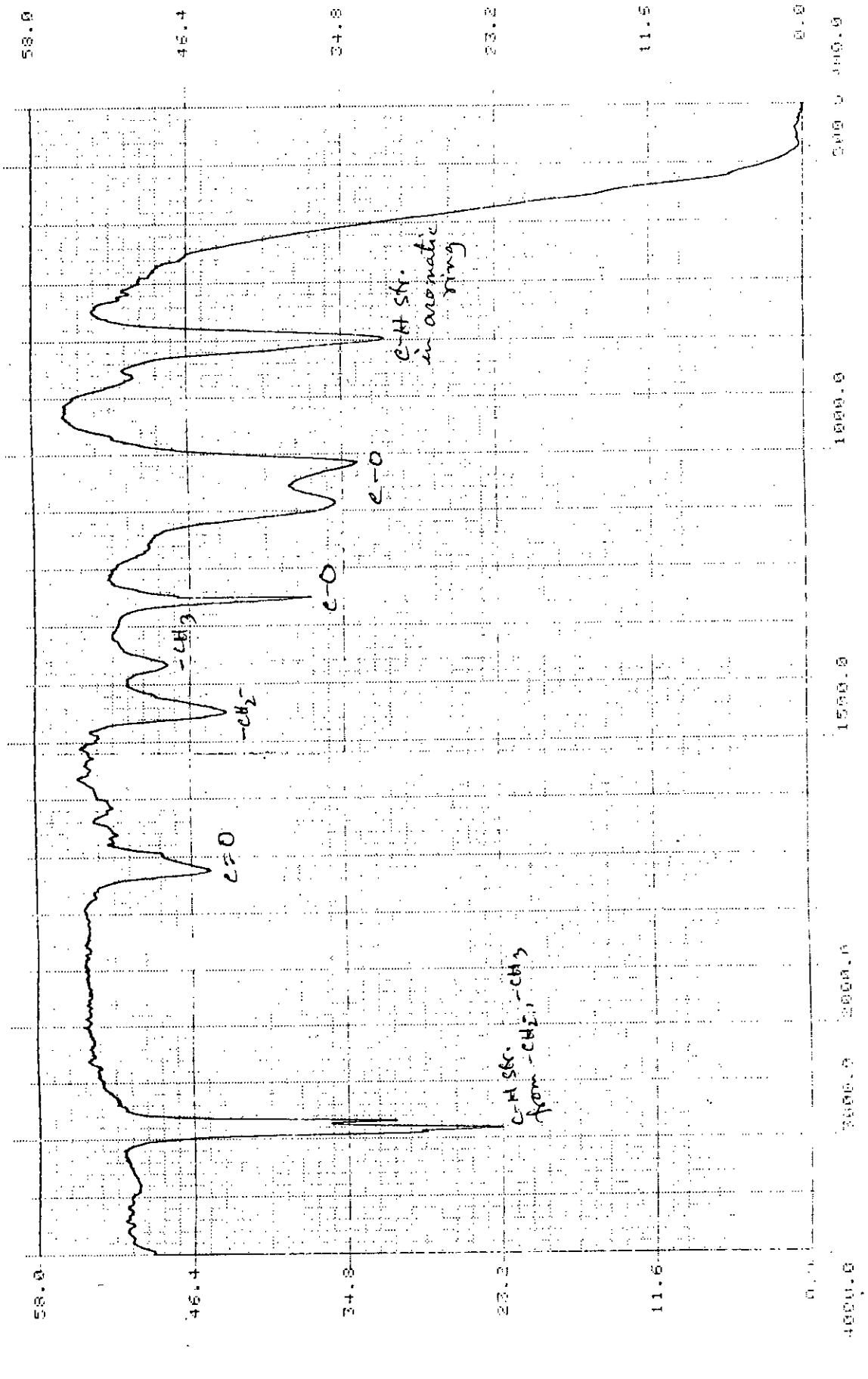


Fig. 4.1 : IR spectrum of LZFA

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treated with boron trifluoride-methanol to prepare their methyl ester by following the method 2.1.6.1 as described in section 2. The ester-derivatives were then subjected to GLC. A comparison of the retention times of these derivatives with those of standard samples revealed the presence of nonanoic acid (minor), undecanoic acid (minor), lauric acid (minor), myristic acid (minor), palmitic acid (major), stearic acid (major), oleic acid (major). However, the chromatograph contained a few other peaks which could not be identified due to lack of the availability of some other standard samples.

4.1.1.1 : CHARACTERIZATION OF LZ1 AS STIGMASTAN-7,22-DI-ENE-3β-OL:

The compound LZ1 was obtained as white crystals from the pet ether (60-80^{0}C) extract of the stems of *Leucas Zeylanica Linn.*. It melted at 157-158^{0}C. It gave a violet colour spot on TLC plate over silica gel when the plate was sprayed with vanillin-sulphuric acid reagent followed by heating in an oven at 110^{0}C for 10 minutes. It gave a positive test towards SalKowski and Liebermann -Burchard reagent showing its steroidal nature which receives support from the bands at 960 and 765 cm^{-1} in the IR spectrum (Figure 4.2). The absorption band at ν 3450 cm^{-1} plus the band at 1060 cm^{-1} was indicative of a hydroxyl group (-OH) in a cyclic system. The sharp absorption bands at ν 2900 and 2850 c^{m-1} together with the absorption bands at ν 1460 cm^{-1} and 1375 cm^{-1} were suggestive of -CH_2- and -CH_3 groups (Williams and Fleming, 1973).

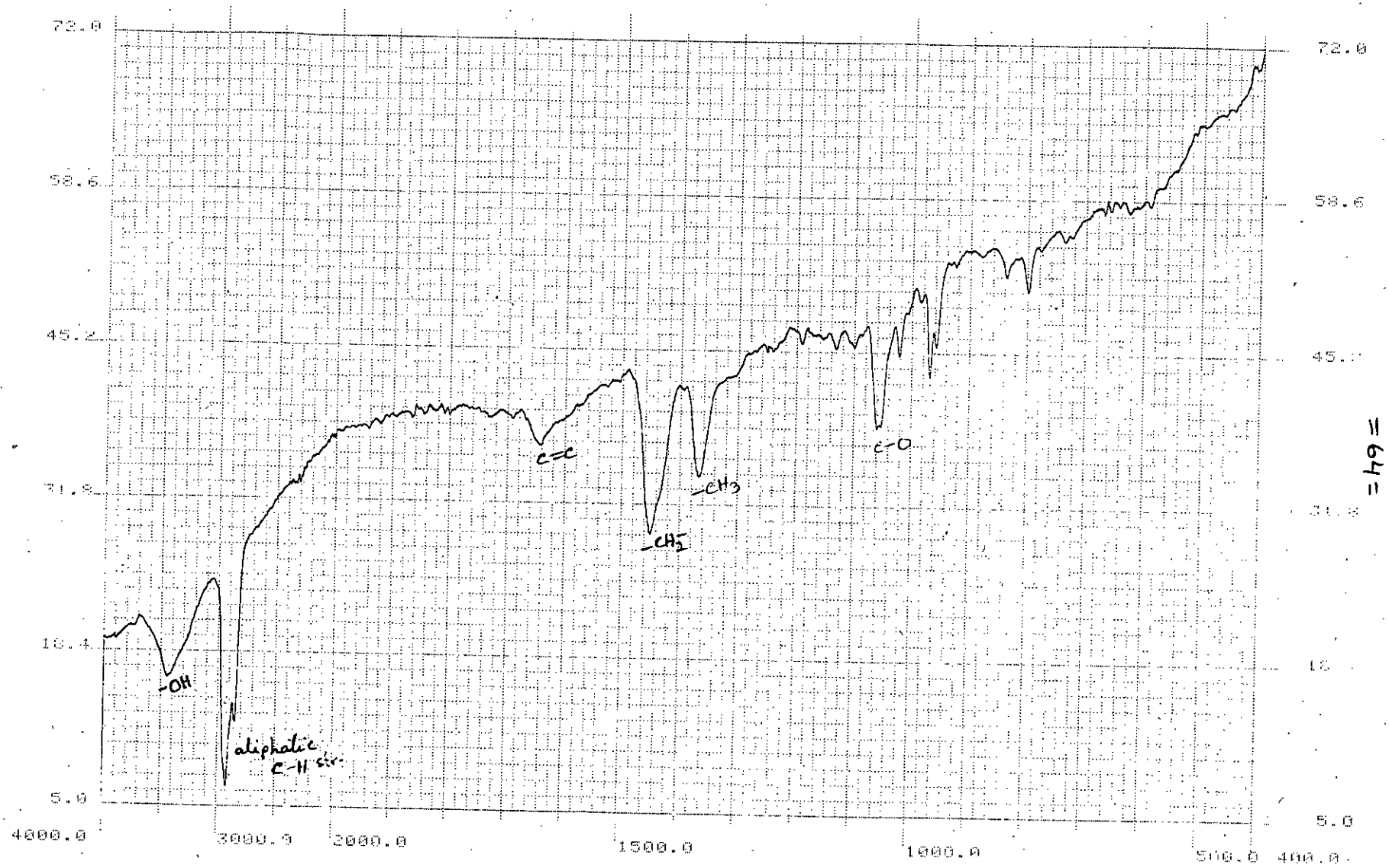

Fig. 4.2 : IR spectrum of LZ1 (22)

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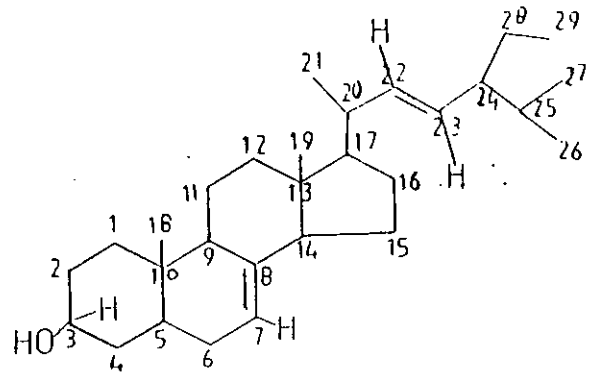
The ^1H NMR spectrum (500 MHz $CDCl_3$, TMS, Figure 4.3) showed three doublets of three protons each at δ 0.657 (J = 5.4), 0.820 (J = 4.5) and 0.768 (J = 5.0) assignable to 21-Me, 26-Me and 27-Me respectively. The doublet of quartet at δ 3.476 (J = 11.72, 10.97, 4.19) was suggestive of an oxymethine proton flanked by two methylene groups of a cyclohexane ring system (**Kobayashi**, 1973) and the higher coupling constant (J = 11.72) was indicative of its β-orientation. Its placement at C-3 of the ring system is supported by the biogenetic ground (Scheme 4, **Ruzicka**, 1953). Two doublets of doublets (dd, 1H each) at δ 4.972 and 5.106 with coupling constants 15.18 and 8.5 are exhibitive of *trans* olefinic proton plus an adjacent methine protons. A 1H broad doublet at δ 5.323 (J = 2.35) was exhibitive of an olefinic proton at C- 7 (**Kobayashi**, 1973).

The ^1H-^1H 2D NMR spectrum (Figure 4.4) showed the correlation between the resonances at δ 4.972 and 5.102 confirming the presence of *trans* olefinic protons at C_{22} and C_{23}. The broad singlet at δ 5.323 showed the connectivities with resonance at δ 1.95 arising from C-6 methylene protons. The resonance at δ 3.476 showed correlations with δ 2.182 (m), 1.932 (m), 1.5 (m) confirming the equivalence of C-2 methylene protons and nonequivalence of C-4 methylene protons. The nonequivalence of C-4 methylene protons is possibly due to asymmetric centre at C-5. On the basis of the foregoing discussions, the compound LZ1 is believed to have the structure (22).

SAMPLE		DEC. & VT	
date	Jul 1 98	dfrq	499.885
solvent	CDCl3	dn	H1
file	exp	dpwr	30
ACQUISITION		dof	0
sfrq	499.885	dm	nnn
tn	H1	dmm	c
at	1.893	dmf	9900
np	22720	dseq	
sw	6000.2	dres	1.0
fb	3000	homo	n
bs	32	temp	27.0
tpwr	57	DEC2	
pw	5.7	dfrq2	0
d1	0	dn2	
tof	255.3	dpwr2	1
nt	8	dof2	0
ct	8	dm2	n
alock	s	dmm2	c
gain	not used	dmf2	200
FLAGS		dseq2	
il	n	dres2	1.0
in	n	homo2	n
dp	y	PROCESSING	
hs	nn	wtfile	
DISPLAY		proc	ft
sp	170.2	fn	not used
wp	3509.7	math	f
vs	224		
sc	0	werr	
wc	250	wexp	
hzmm	14.04	wbs	
is	33.57	wnt	wft
rfl	263.4		
rfp	0		
th	7		
ins	1.000		
nm cdc ph			



(22)

Fig. 4.3 : ^{1}H NMR spectrum of LZ1 (22)

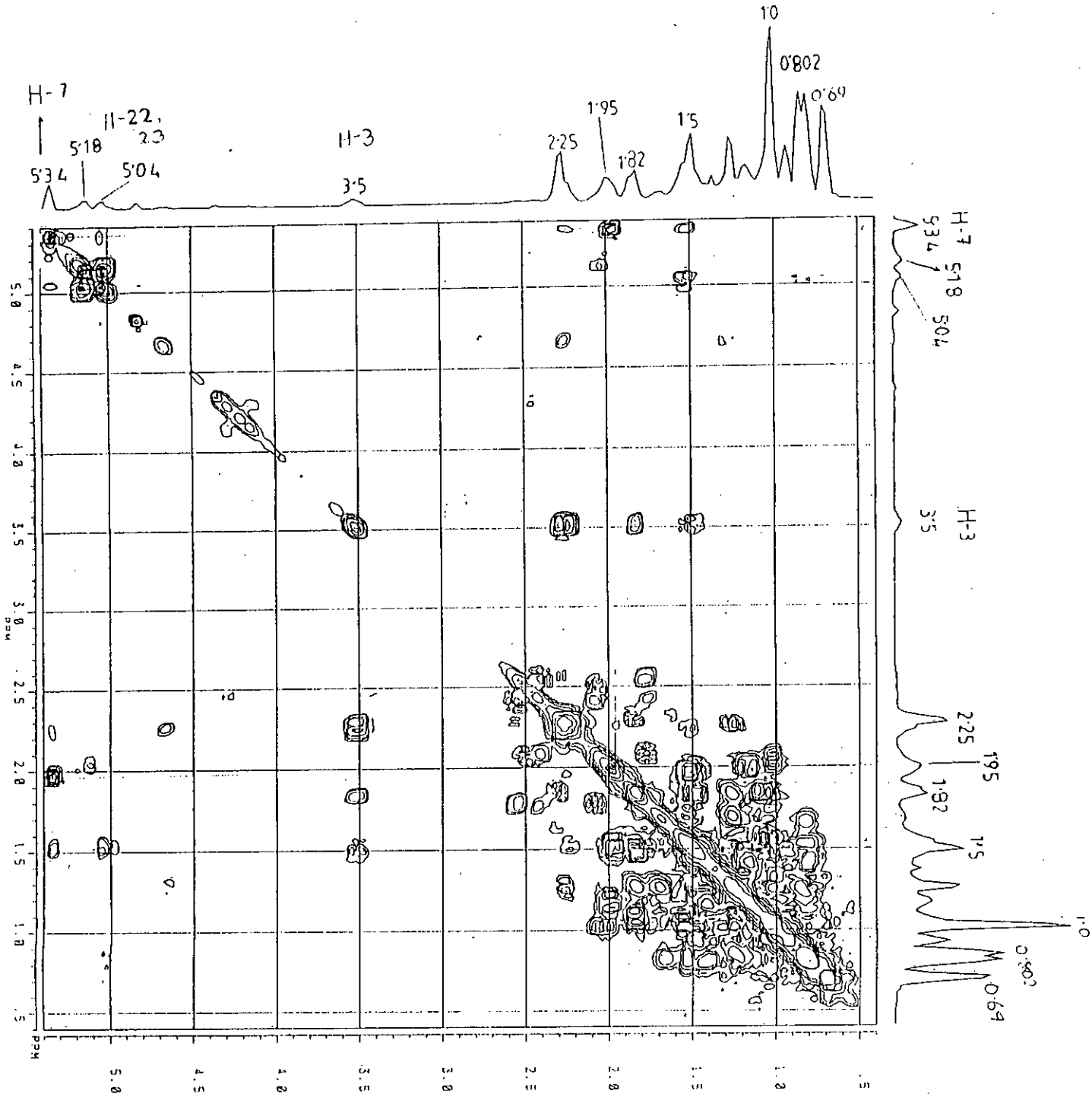
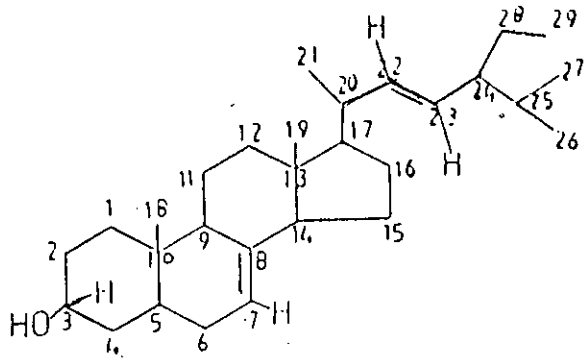


Fig. 4.4 : 2D ^{1}H-^{1}H NMR spectrum of LZ1 (22)

The presence of the double bonds at C-7 and C-22 receive further support from the mass peak at m/e 271 formed by the allylic cleavage and m/e 300 arising from the retro-Diels-Alder fragmentation process (Wyllie,1968). The other mass spectral fragments (Scheme 5, Figure 4.5) are compatible with those of the identified structure (22) for LZ1. It was named as Stigmastan-7,22-di-ene-3-β-ol (22).



(22)

The isolation of LZ1 and its identification to have the structure(22) constitute the first report of its occurrence in this plant. Its biosynthesis in this plant is in conformity with the report of isolation from other plants belonging to the same genus (Misra et al. 1992).

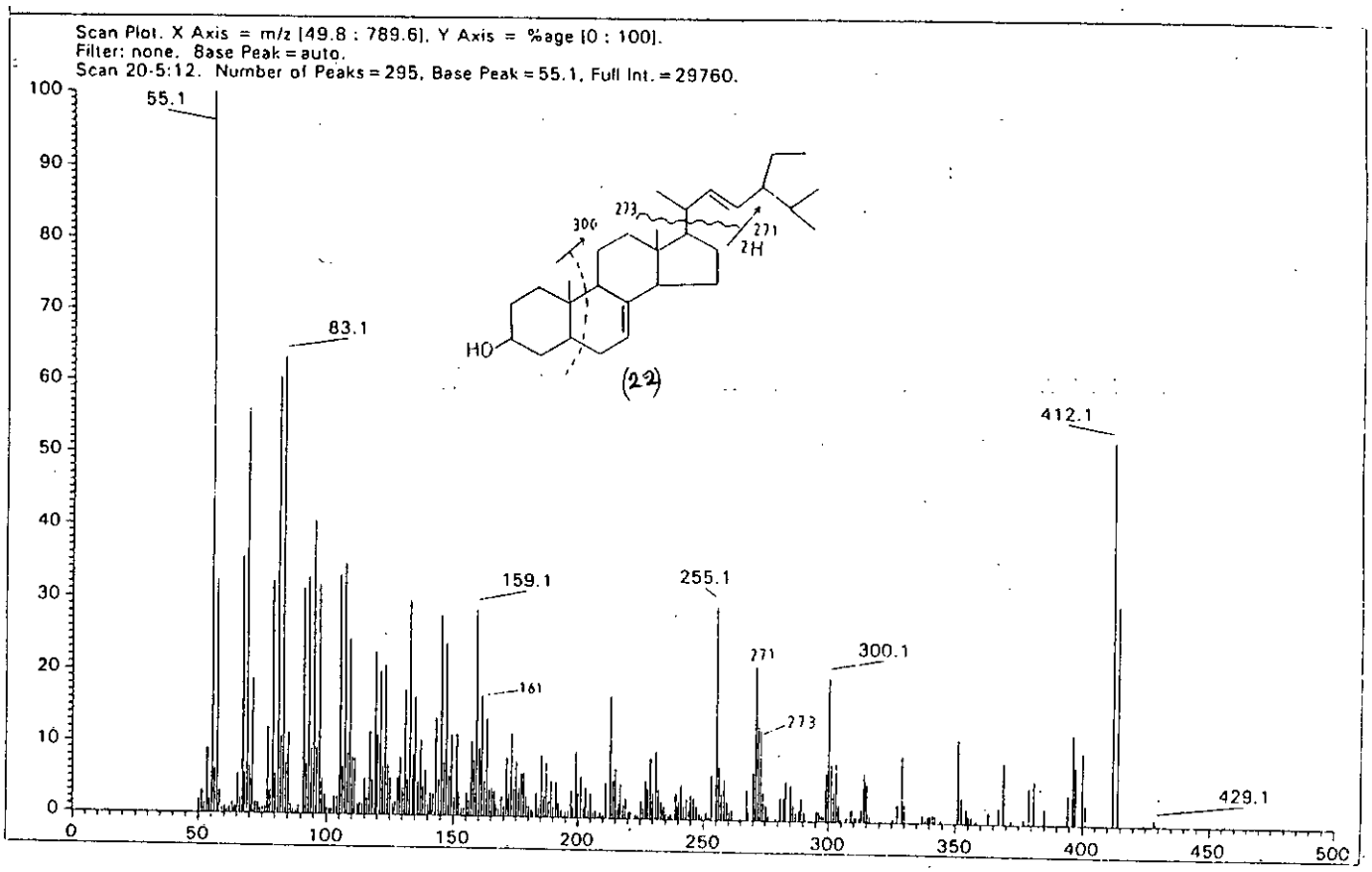
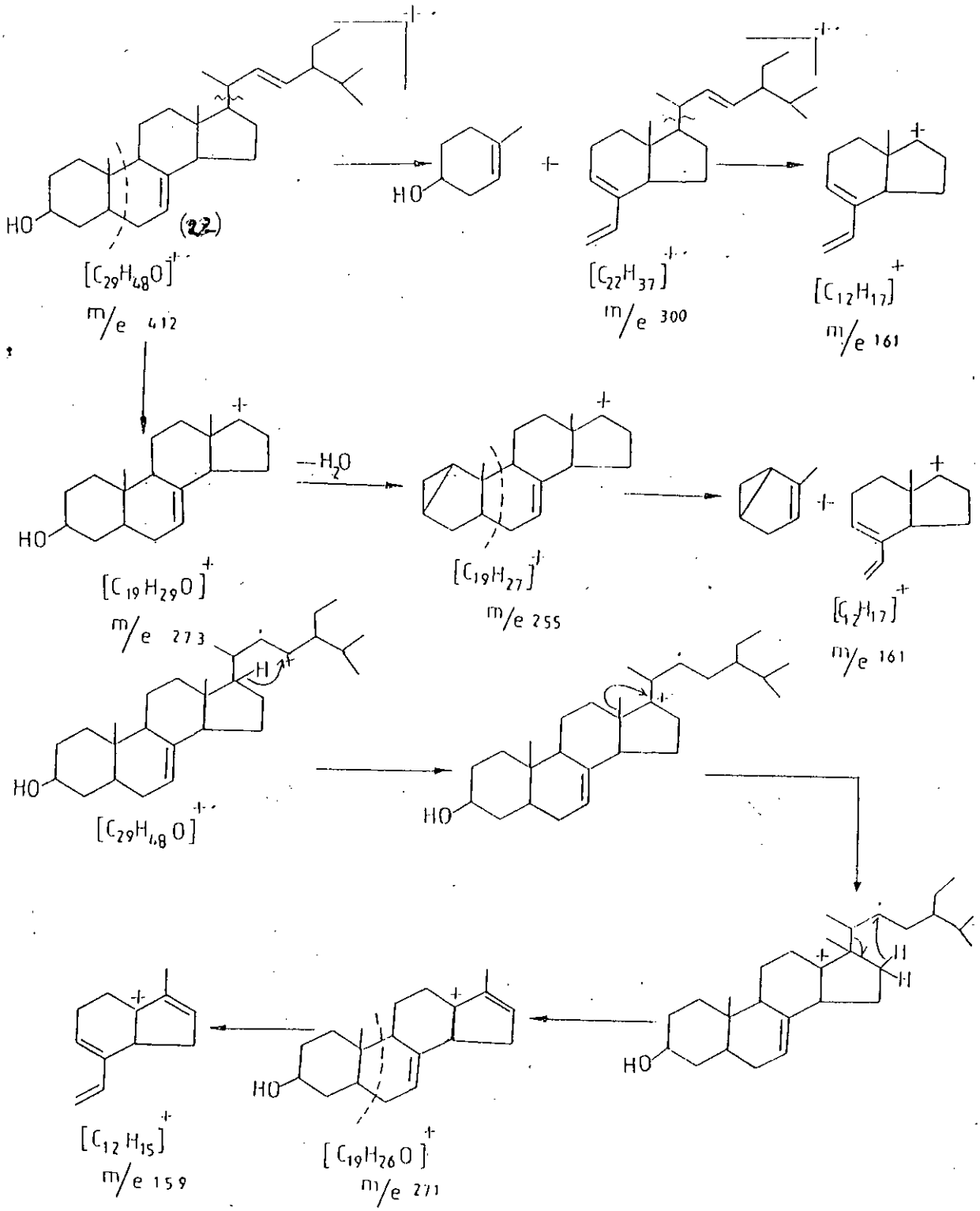


Fig. 4.5 : EIM spectrum of LZ1 (22)



Scheme[4] Possible mass fragmentation of (22)

4.1.1.2 : CHARACTERIZATION OF LZ2 AS 28-EPOXY-STIGMAST-7, 22- DI-ENE- 3β-*O*-GLUCOSIDE :

LZ2 was obtained as an ash colour amorphous powder from the EtOAc extract of the stems of *Leucas Zeylanica Linn.*. It melted at 294-295^{0}C. It gave a blue fluorescing spot on TLC plate over silica gel PF_{254} under UV light at 366 nm. It also produced a violet colour spot when a silica coated TLC plate was sprayed with vanillin-sulphuric acid followed by heating in an oven at 110^{0}C for 10 minutes. It responded to the Salkowski test and Liebermann-Burchard`s test showing its steroidal nature and it also responded to Phenol-sulphuric acid test confirming the presence of a sugar moiety in it. Furthermore, these qualitative tests indicate that the compound LZ2 might be a steroidal glycoside.

Its IR spectrum (Figure 4.6) showed a strong absorption band at ν 3400 cm^{-1} indicative of a hydroxyl group (-OH). The sharp absorption bands at ν 2900 cm^{-1}, 2880 cm^{-1} along with the band at ν 1370 cm^{-1}, 1450 cm^{-1} were demonstrative of $-CH_2-$ and $-CH_3$ groups. (**Williams and Fleming**, 1973). The bands at ν 1250 and 1050 cm^{-1} are due to C-O stretching and suggestive of either an epoxide ring or a glycosidic linkage or both. In addition to this, the bands at ν 970 and 960 cm^{-1} are indicative of its steroidal nature (**Finar**, 1982). A number of tiny bands both at ν 1620 and 730 cm^{-1} were taken as indications of the presence of a C=C bond in the compound LZ2.

Fig. 4.6 : IR spectrum of compound LZ2 (23)

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The ^{1}H NMR spectrum (500 MHz, deuterated pyridine , Figure 4.7) with a bunch of signals within δ 3.889-4.487 and δ 1.00-2.00 were indicative of a steroidal glycoside molecule. A singlet at δ 0.868 integrating for six protons were ascribable to two angular methyl protons H-18 at δ_c 12.01 and H-19 at δ_c 12.71. Four doublets at δ 1.001 (J=7.14 Hz), 0.911 (J=2.4 Hz), 2.0 (J=2.5 Hz) and 0.815 (J=2.00 Hz) each integrating for three protons could be assigned to Me-21 at δ_c 12.55, Me-26 at δ_c 19.25, Me-29 at δ_c 123 and Me-27 at δ_c 21.50 respectively. The down field 1H broad singlet at δ 5.277 was demonstrative of an olefinic proton H-7 at δ_c 29 (Tessier et al., 1982). One doublet of doublet centered at δ 4.220 (J= 3.5, 3.41 Hz) and a triplet at δ 3.992 (J = 3.2,3.3 Hz) integrating one proton and two protons revealed that H-3' at δ_c 78.51 and H-4' at δ_c 71.75 and H-6b'protons respectively. A clear doublet at δ 4.982 (J=7 Hz) is assignable to an anomeric proton H-1' at δ_c 102 (Quader. et. al. 1990). The doublet of doublet at δ 4.00 (J= 2.5, 2.54) integrating one proton was suggestive of H-2'proton at δ_c 78.64. The broad doublet at δ 4.337 (1H, d, J = 6.5 Hz) and at δ 4.40 (1H, d, J = 4.3 Hz) were indicative of H-5'at δ_c 78.14 and H-6a' at δ_c 62.89 proton respectively. One proton doublet of doublet centered at δ 5.126 (J = 12.5, 5.8 Hz) which could be attributed to the olefinic proton of H-22 at δ_c 121. One proton doublet at δ 4.983 (J = 12.5, 6.5 Hz) was indicative of H-23 proton at δ_c 135.81. The triplet centered at δ 2.41 (J= 5.1, 6.0 Hz) for one proton was indicative of H-28 proton at δ_c 150.24 and another singlet centered at δ 4.85 of one proton intensity was

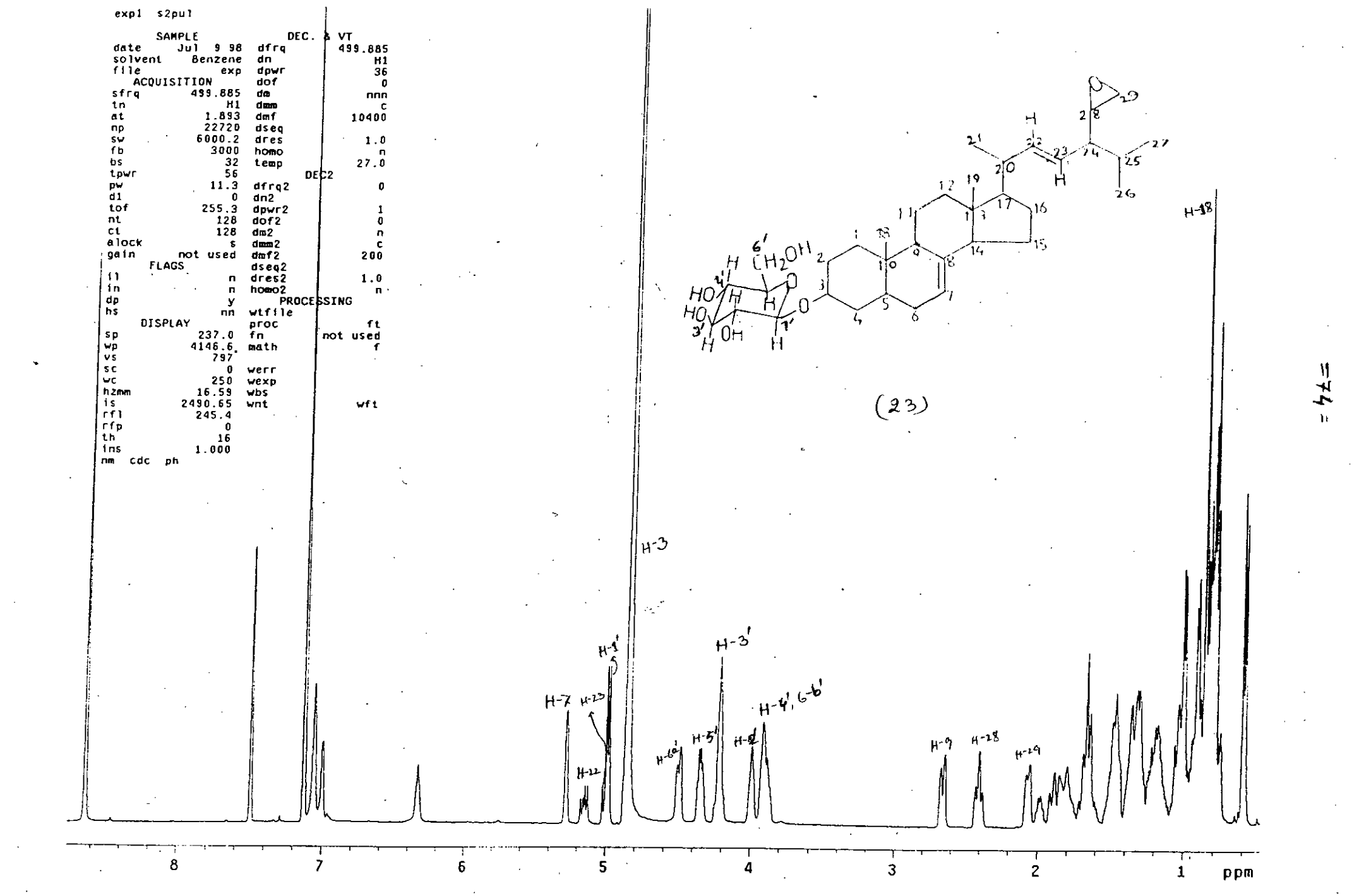


Fig. 4.7 : ^{1}H NMR spectrum of LZ2 (23)

assignable to H-3 proton. The chemical shift and coupling constant suggested the β-linkage of the sugar moiety (Bina et al.,1994).

The 2D NMR spectrum (deuteropyridine, Figure 4.8) showed a broad singlet at δ 5.277 which exhibited connectivity to a multiplet for H-6 at δ 1.8. The doublet of doublet at δ 5.126 showed a clean correlation with the doublet of doublet at δ 4.982. Thus the protons H-22 and H-23 labelled in the 2D NMR spectrum have been conclusively assigned.The doublet at δ 4.982 (H-1' anomeric, J=7 Hz) showed the connectivity with the resonance at δ 4.00 for H-2' which is, in turn, exhibited a correlation with H-3' at δ 4.220. This H-3' exhibited a relation with a triplet like peak at δ 3.992 for H-4' which is again connected with a broad doublet at δ 4.337 for H-multiple'. This H-5' peak is related with the peak at δ 3.992 for H-6b' that is also in connection with H-6a'at δ 4.40. Thus, the connectivity of the oxymethine protons along with the anomeric proton in the sugar molecule has been conclusively established. The singlet at δ 4.85 was assignable to H-3 oxymethine proton to which a glucose moiety is linked up. The connectivity of the bunches of signals in the region within δ 0.6-2.5 could not be ascertained clearly. Even then, the expanded 2D-NMR spectra (Figure 4.8.1 and 4.8.2) have been depicted here for its clarity.

The ^{13}C-NMR spectrum (deuteropyridine, Figure 4.9) showed signals for 35 carbons. The anomeric carbon signal is centered at δ_c 102 (Quader et al., 1990). The other carbon signals have been

AR-L2-cost
Solvent: Benzene
Temp. 27.0 C / 300.1 K
INOVA-500 "Deadhead3"
PULSE SEQUENCE: cosy
Relax. delay 1.000 sec
Acq. time 0.244 sec
Width 4201.9 Hz
2D Width 4201.9 Hz
4 repetitions
256 increments
OBSERVE H1, 499.8821499 MHz
DATA PROCESSING
Sine bell 0.122 sec
F1 DATA PROCESSING
Sine bell 0.030 sec
FT size 2048 x 2048
Total time 22 minutes

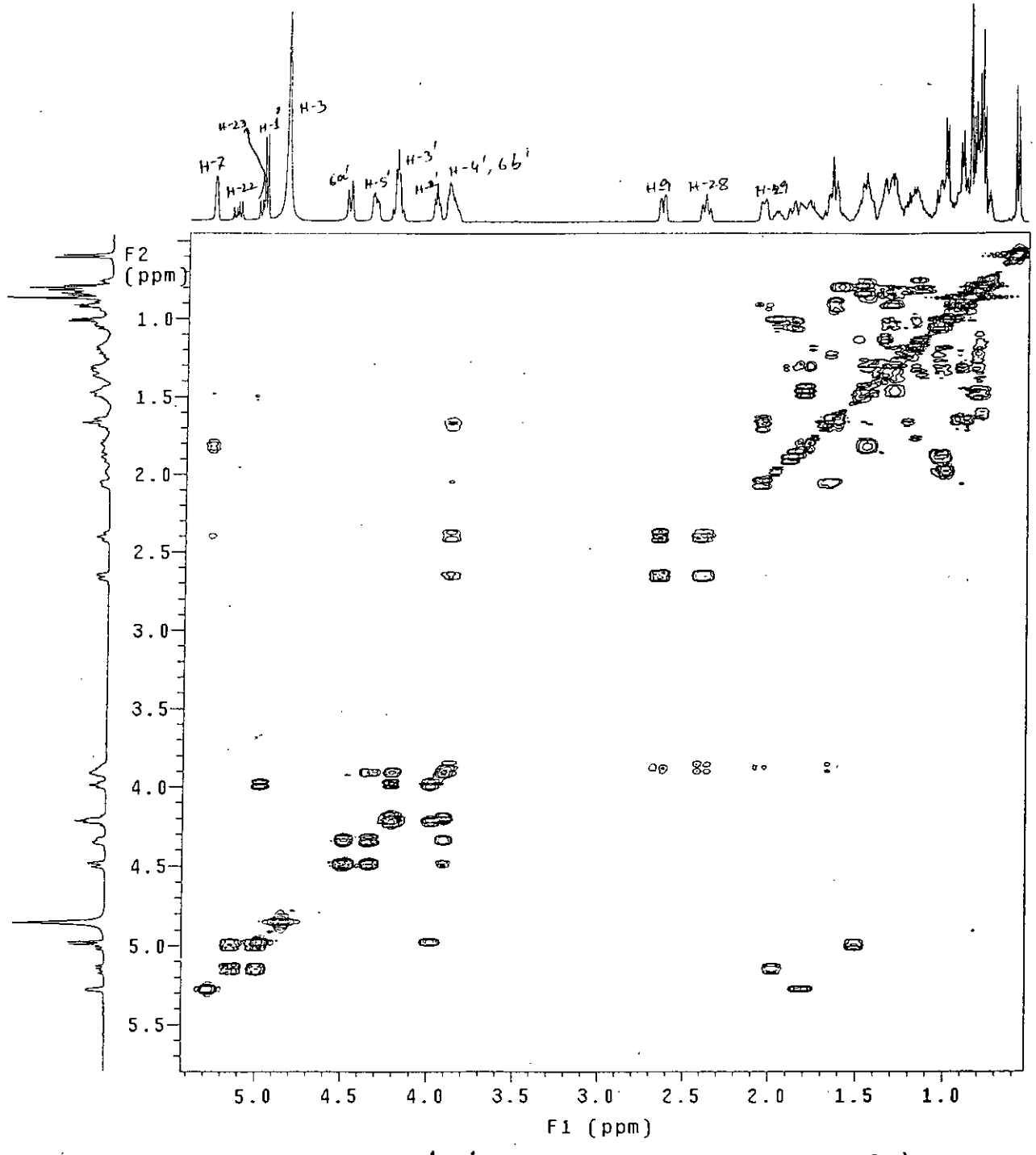


Fig. 4.8 : 2D ^{1}H-^{1}H NMR spectrum of LZ2 (23)

AR-L2-cost

Solvent: Benzene
Temp. 27.0 C / 300.1 K
INOVA-500 "Deadhead3"

PULSE SEQUENCE: cosy
Relax. delay 1.000 sec
Acq. time 0.244 sec
Width 4201.9 Hz
2D Width 4201.9 Hz
4 repetitions
256 increments
OBSERVE H1, 499.8821499 MHz
DATA PROCESSING
Sine bell 0.122 sec
F1 DATA PROCESSING
Sine bell 0.030 sec
FT size 2048 x 2048
Total time 22 minutes

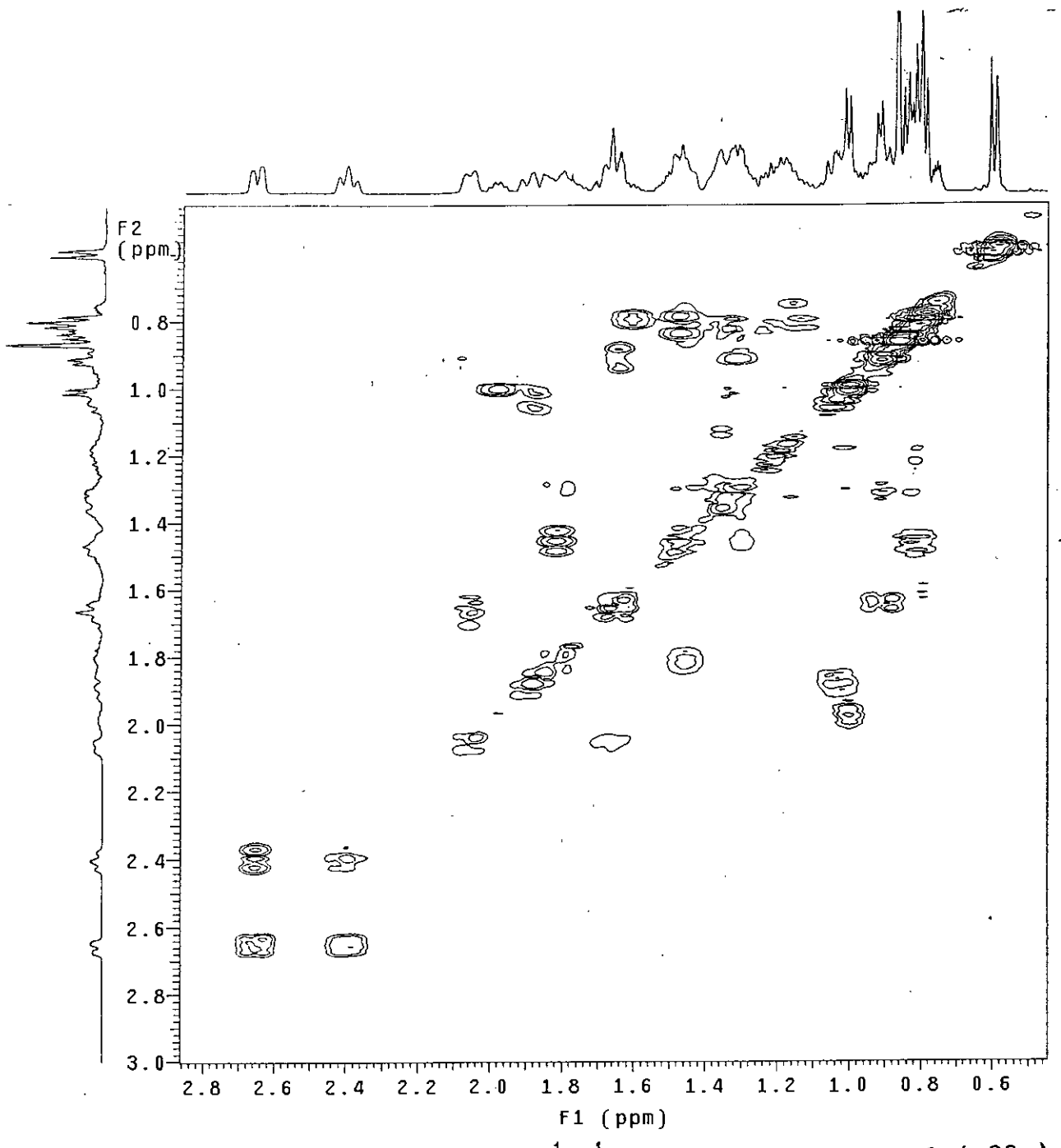


Fig. 4.8.1: Expanded 2D ^{1}H-^{1}H NMR spectrum of LZ2 (23)

Aug 21 98 Benzene AR-L2-cost

AR-L2-cost
Solvent: Benzene
Temp. 27.0 C / 300.1 K
INOVA-500 "Deadhead3"
PULSE SEQUENCE: cosy
Relax. delay 1.000 sec
Acq. time 0.244 sec
Width 4201.9 Hz
2D Width 4201.9 Hz
4 repetitions
256 increments
OBSERVE H1, 499.8821499 MHz
DATA PROCESSING
Sine bell 0.122 sec
F1 DATA PROCESSING
Sine bell 0.030 sec
FT size 2048 x 2048
Total time 22 minutes

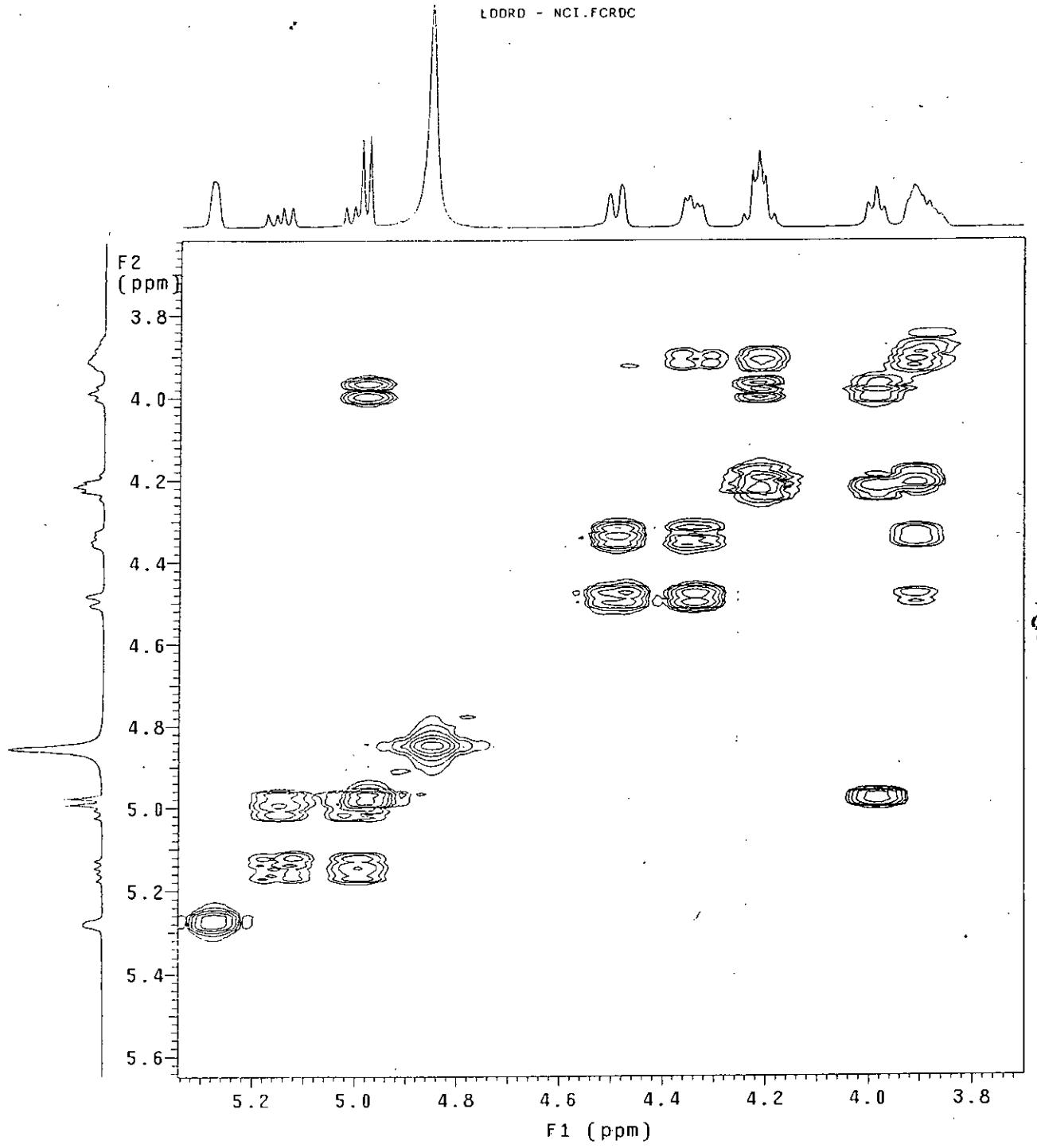


Fig. 4.8.2: Expanded 2D ^{1}H-^{1}H NMR spectrum of LZ2 (23)

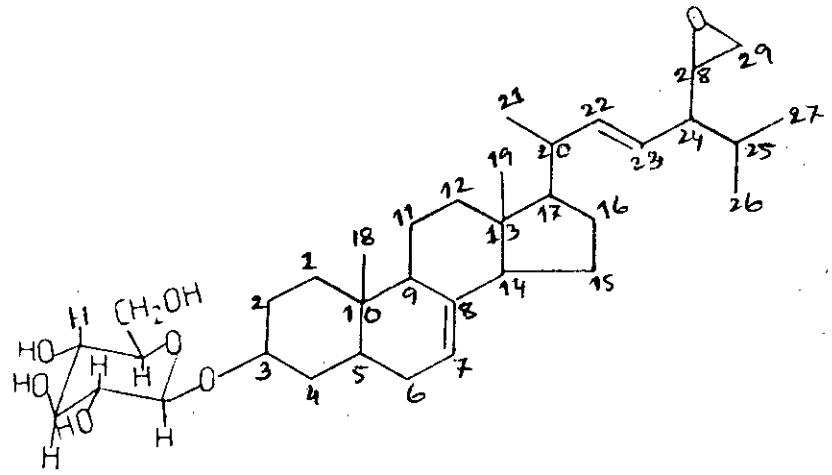
Jul 20 98 AR-L2-C13

LDDRD - NCI.FCRDC

AR-L2-C13 Pyridine

exp2 s2pul

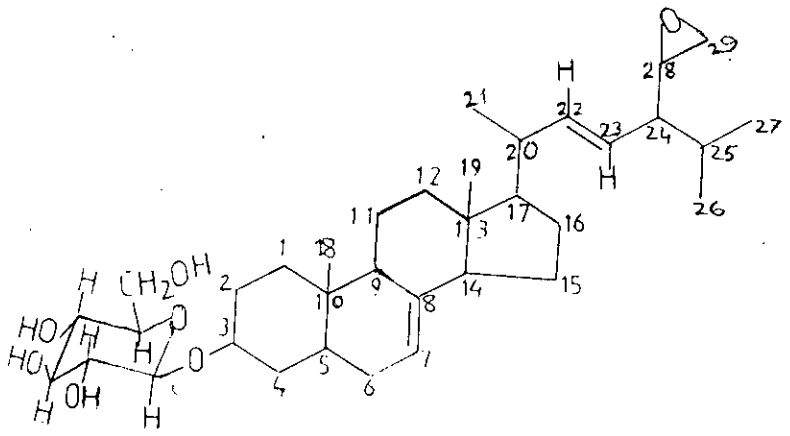
SAMPLE		DEC. & VT	
date	Jul 20 98	dfrq	499.885
solvent	Benzene	dn	H1
file	exp	dpwr	36
ACQUISITION		dof	0
sfrq	125.709	dm	yyy
tn	C13	dmm	g
at	1.300	dmf	10400
np	75200	dseq	
sw	28912.2	dres	1.0
fb	16000	homo	n
bs	64	temp	27.0
tpwr	56	DEC2	
pw	7.3	dfrq2	0
d1	0	dn2	
tof	1869.5	dpwr2	1
nt	24744	dof2	0
ct	24744	dm2	n
alock	s	dmm2	c
gain	not used	dmf2	10000
FLAGS		dseq2	
il	n	dres2	1.0
in	n	homo2	n
dp	y	PROCESSING	
hs	nn	lb	0.50
DISPLAY		wtfile	
sp	-446.2	proc	lp
wp	28912.2	fn	not used
vs	113	math	f
sc	0		
wc	250	werr	
hzmm	115.65	wexp	
is	500.00	wbs	
rfl	15969.5	wnt	
rfp	15523.4		
th	68		
ins	1.000		
nm	ph		



(23)

labelled in the spectrum and also in the structure of the compound LZ2.

In the light of the foregoing discussion (IR, ^{1}H-NMR, ^{13}C-NMR and 2D-NMR) the compound LZ2 has been identified as 28-EPOXY STIGMAST-7,22-DI-ENE-3β-*O*-GLUCOSIDE (23).



(23)

4.1.1.3 : CHARACTERIZATION OF LZA2 (ACETYL DERIVATIVE OF LZ2):

LZA2 was obtained as a light yellow crystalline compound from LZ2 by acetylation . Its melting point was 150-51^{0}C. It was viewed as a bluish spot on TLC plate over silica gel PF254 (EtOAC : Pet

ether = 4 : 1) under UV light (366 nm). It turned into a pinkish spot on spraying with 1% vanillin-sulphuric acid followed by heating at 110°C for 10 minutes. It responds to the SalKowski and Liebermann-Burchard`s test showing its steroidal nature and it also gave positive response towards Phenol sulphuric acid test showing the presence of a sugar moiety in it.

The IR spectrum (Figure 4.10) of LZA2, the acylated product of LZ2 showed an absorption peak at ν 1740 cm^{-1} (Which was not present in the IR spectrum of LZ2 (Figure 4.6), and at the same time the hydroxyl peak at 3400 cm^{-1} of LZ2 has been reduced to a great extent indicating the acetylation of hydroxyl group/ groups of the sugar molecule. Its ^1H NMR spectrum (60 MHz, $CDCl_3$, TMS, Figure 4.11) showed signals for alkyl groups within δ 0.5-1.7 indicating its steroidal identity. Furthermore, the spectrum showed signals at δ 2.00 attributable to acyl groups which are absent in the ^1H-NMR spectrum of LZ2. The signals within δ 4.0-5.2 are due to oxymethine, epoxide protons and H-3 proton.

The IR and ^1H-NMR data of the acylated derivative LZA2 of LZ2 lend further supports towards the structure (23) of LZ2.

Although the isolation of a steroidal glycoside has been reported from some of the species of the genus Leucas, a steroidal glucoside ornamented with an epoxide ring has not yet been reported. So, the isolation of this compound is expected to constitute a new report. It is named as 28-epoxy-stigmast-7, 22-di-ene-3β-*O*-glucopyranoside (23).

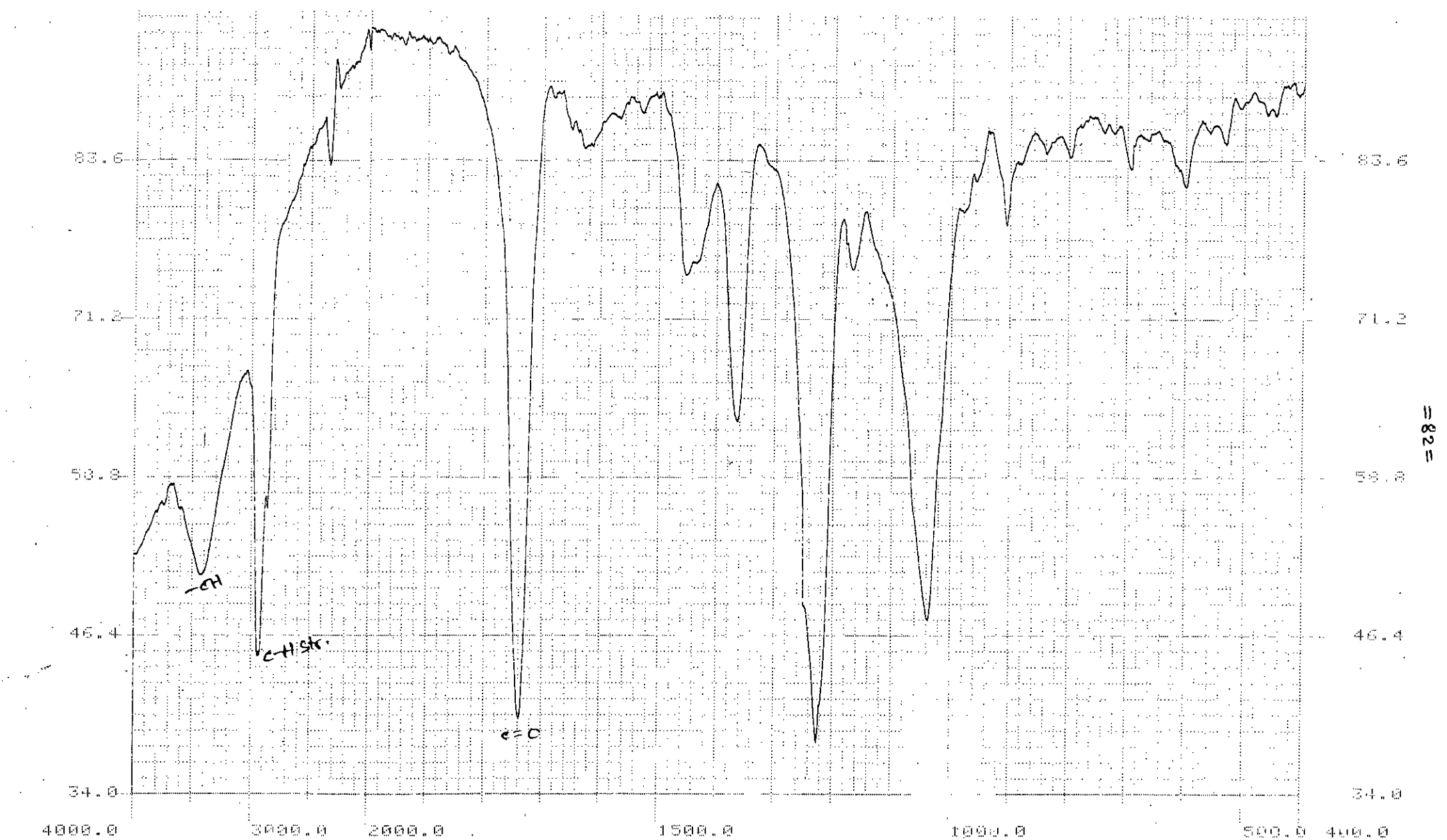


Fig. 4.10 : IR spectrum of LZA2 (acetylated compound)

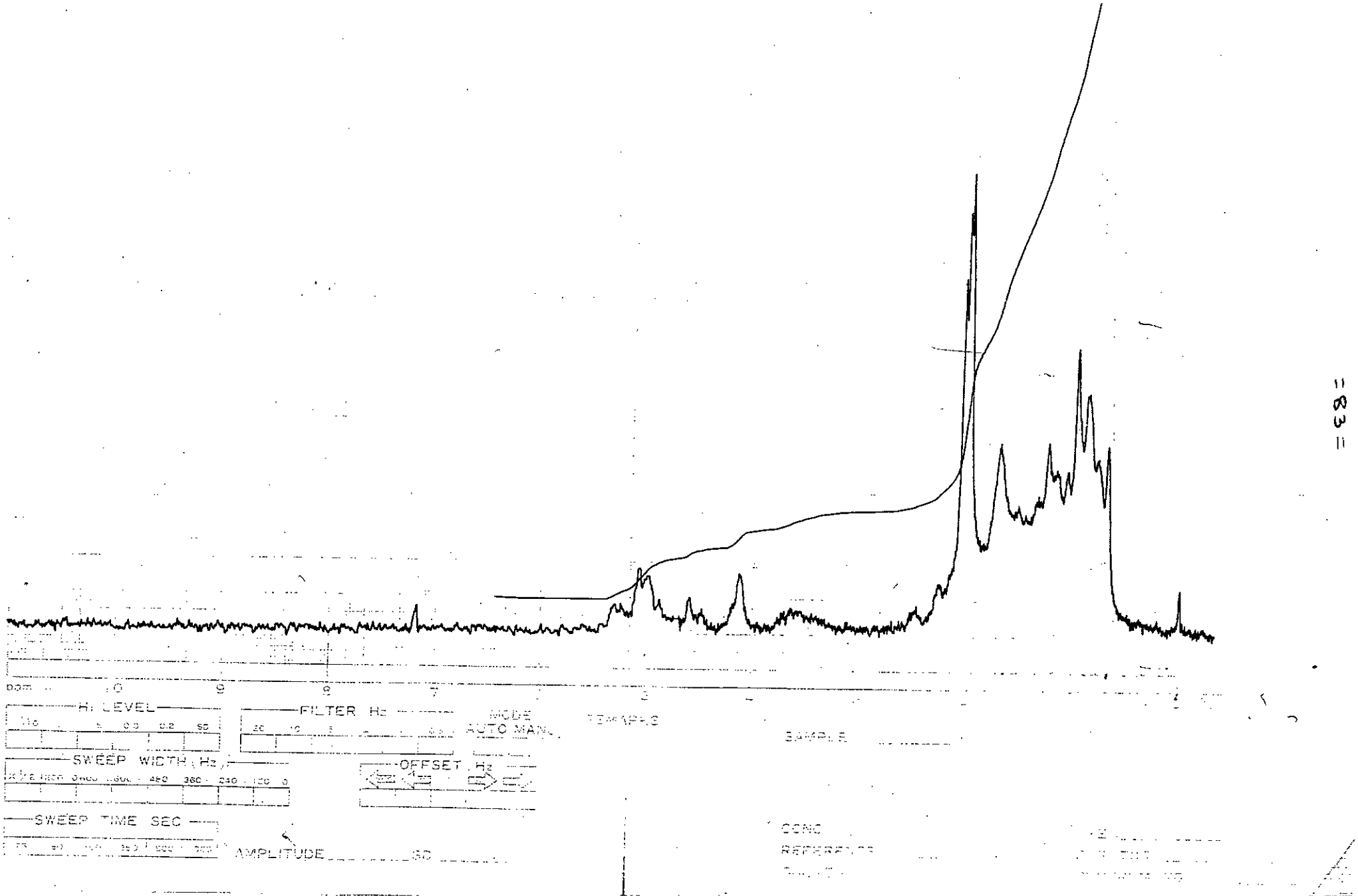


Fig. 4.11 : ^{1}H NMR spectrum of LZA2

4.1.1.4 : CHARACTERIZATION OF LZ3 :

The compound LZ3 was isolated as an amorphous brown coloured substance from VLC fractionation of EtOAc extract of the stems of *Leucas Zeylanica Linn*..It melted at 274-275^{0}C. Its chromatogram on a silica gel coated TLC plate (Rf value = 0.65, EtOAC : Pet ether = 5:1) gave a single spot and was visualized as a yellowish brown spot in iodine vapour and this was also observed when it was sprayed with vanillin-sulphuric acid followed by heating in an oven at 110^{0}C for 10 minutes. Like LZ2, it responded to the Salkowski test and Liebermann-Burchard`s test showing its steroidal nature and also to Phenol-sulphuric acid test confirming the presence of a sugar moiety in it. Thus, these qualitative tests again give us the preliminary idea that the compound LZ3 might be a steroidal glycoside like LZ2.

Its IR spectrum (Figure 4.12) is very much similar to that of LZ2 with an exception to the bands at ν 1610 and 1500 cm-1. The ^{1}H NMR spectrum (Figure 4.13) of LZ3 also contains two more signals at δ 2.5 and 3.75. These suggest that this compound may be a stereoisomer of LZ2(23). Its confirmation may only come from NOE experiments.

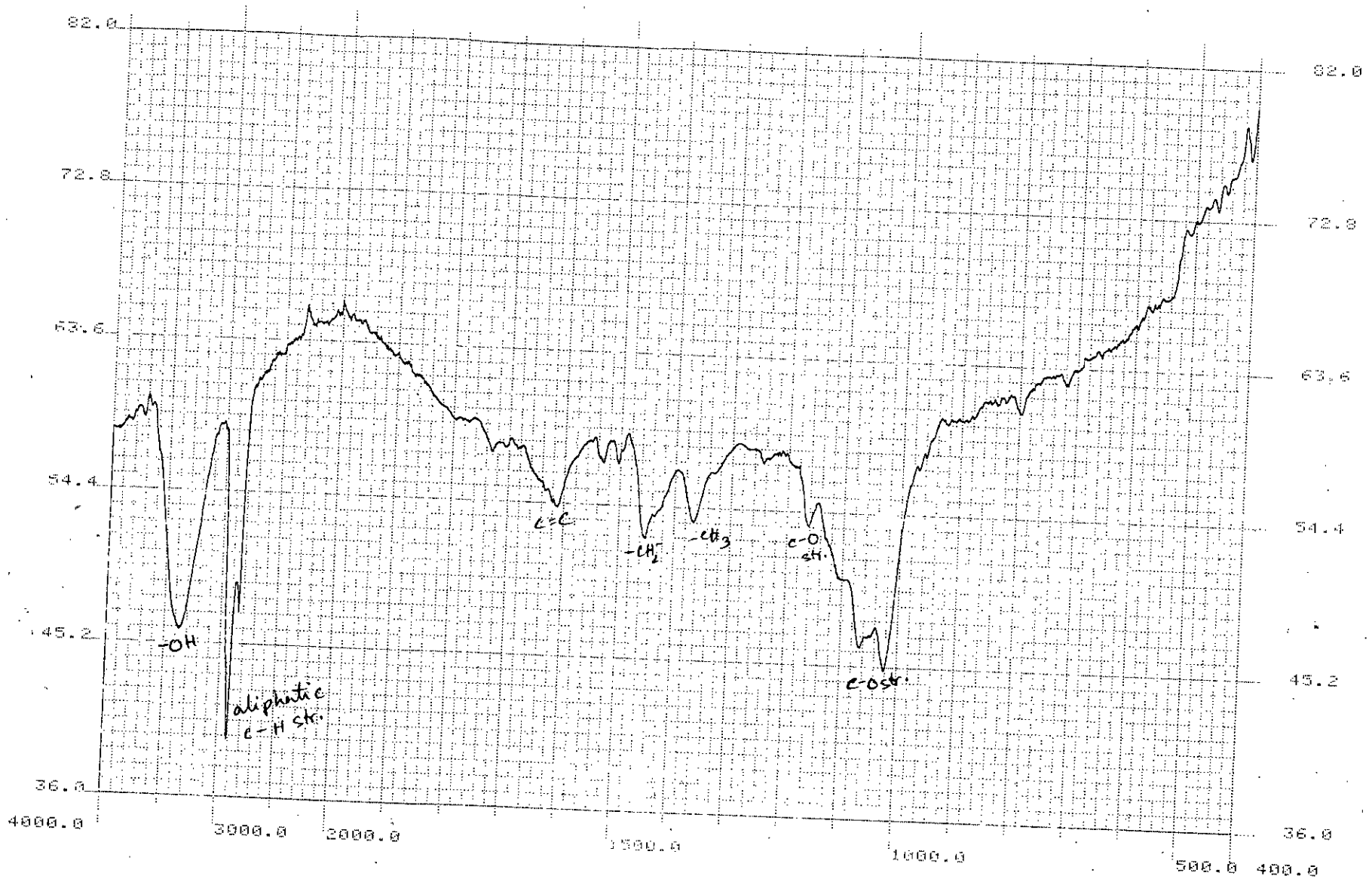


Fig. 4.12 : IR spectrum of LZ3

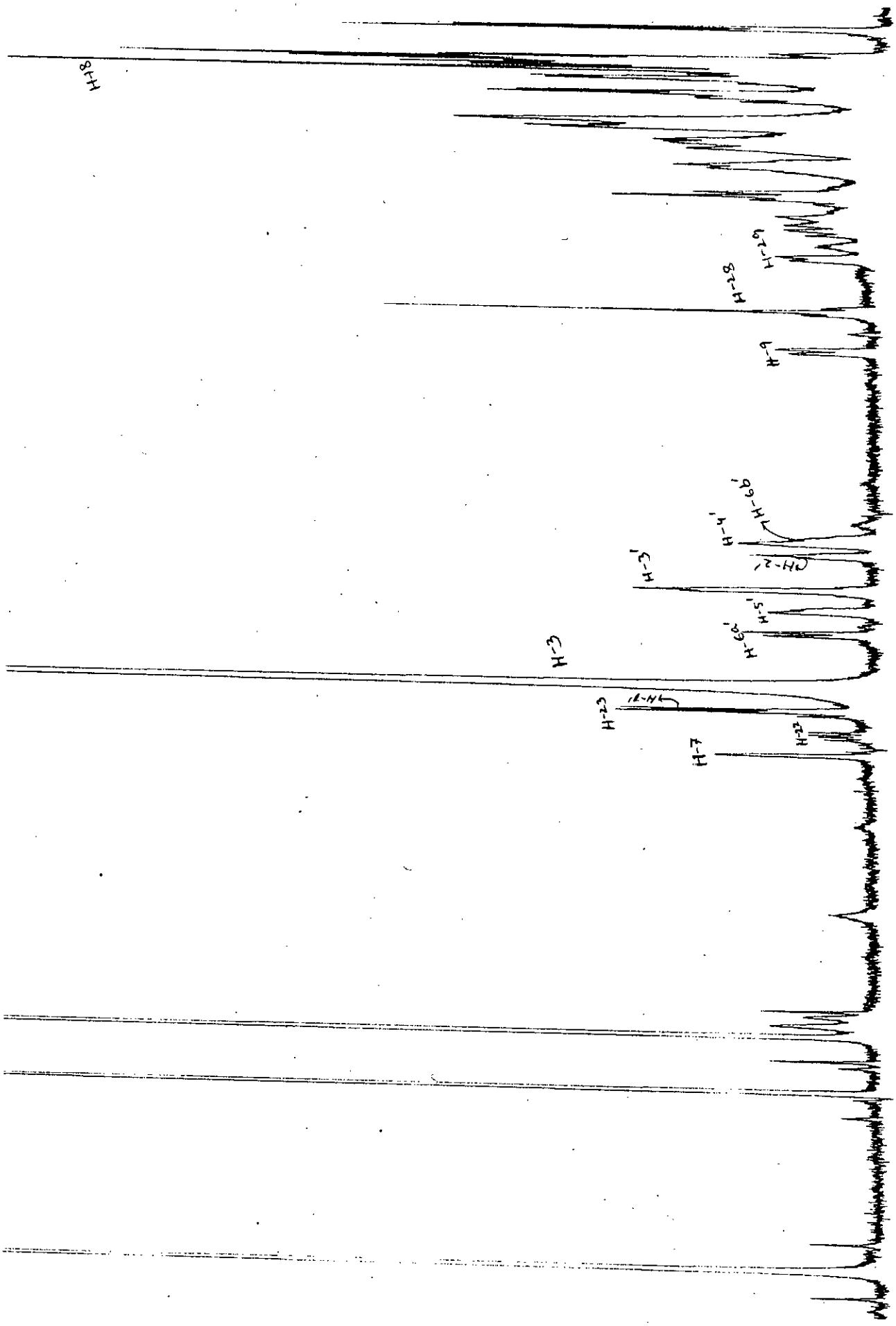


Fig. 4.13 : ^{1}H NMR spectrum of LZ3

4.1.1.5 : CHARACTERIZATION OF LZ4

The compound LZ4 was isolated as a white crystalline substance from VLC fractionation of EtOAc extract of the stems of *Leucas Zeylanica Linn.*. Its melting point was ~ 300^{0}C. It gave a bluish spot on silica coated TLC plate under UV at 366 nm. It produced a pinkish colour spot on silica coated TLC plate when it was sprayed with vanillin-sulphuric acid followed by heating in an oven at 110^{0}C for 10 minutes. It responded to phenol-sulphuric acid test showing the presence of a sugar moiety in it. In contrast to the compounds- LZ1, LZ2 and LZ3, it did not respond to the Salkowski and Liebermann-Burchard`s tests. This is taken as an indication that the compound does not belong to steroidal class of compounds.

Its IR spectrum (Figure 4.14) showed an absorption band at ν 3400 cm^{-1} indicative of a hydroxyl group (- OH). The absorption bands at ν 2900 cm^{-1} and at ν 2850 cm^{-1} are due to C-H stretching arising from -CH_2- and -CH_3 groups. The bands at ν 1610 cm^{-1} were suggestive of a C=C bond enriched moiety. The band at 1450 cm^{-1} is attributable to - CH2- scissoring.The broad absorption band at ν 1080 cm was indicative of CH-OH stretching in the cyclic system.The band at ν 800 cm^{-1} was demonstrative of a -CH=CH- moiety. The bands at 660 cm^{-1} and ν 460 cm^{-1} were suggestive of C-OH and C-O-C bending vibration in the compound.

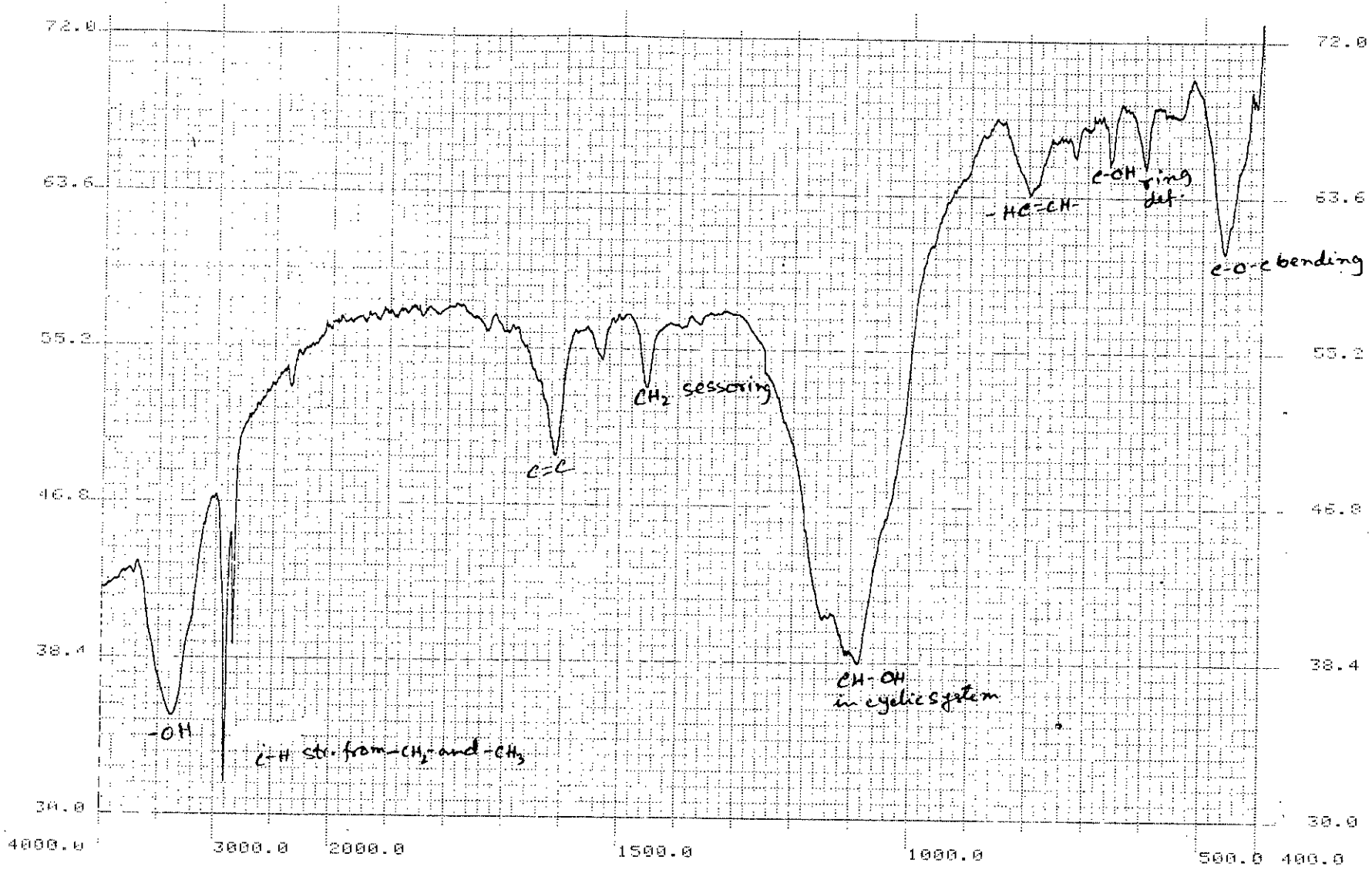


Fig. 4.14 : IR spectrum of compound LZ4 (24)

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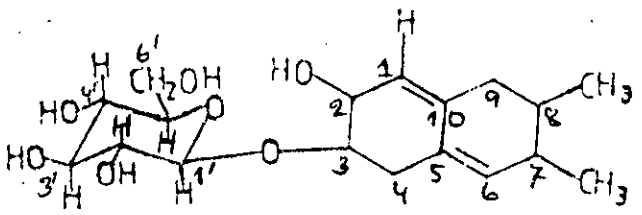
The ^{1}H NMR spectrum (500 MHz, deuteropyridine , Figure 4.15) showed a downfield doublet at δ 8.497 (1H, d, J = 10.25) assignable to H-1 proton. This down field resonance is thought to be due to the deshielding effect arising from its nearby hydroxyl group and anisotropic effect coming from the carbon carbon double bond (-C=C-). A doublet of doublet centered at δ 5.435 (J = 2.5, 2.8 Hz) integrating for one proton was ascribable to an anomeric proton (H-1, Quader et al., 1990). A broad singlet at δ 5.042 (J = 2.9 Hz)integrating for one proton was attributable to 6-b' proton at δ_c 62.07.The broad singlet at δ 4.874 was assignable to hydroxyl groups. Four multiplets centered at δ 4.546, 4.434,4.272 and 4.221 each integrating for one proton were suggestive of H-2' at δ_c 87.64,H-4'at δ_c 72.48,H-3' at δ_c 76.82 and H-6a' at δ_c 62.07 respectively. A multiplet centered at δ 4.344 integrating for one proton was indicative of H-5' proton at δ_c 75.55. A singlet at δ 2.41 (1H, s) was assignable to 2-OH proton.One doublet of doublet at δ 2.19 (J=3.5 Hz) integrating for one proton was suggestive of H-3 proton at δ_c 96.07. One proton multiplet centered at δ 2.136 was suggestive of H-2 proton at δ_c 73.03. Two broad singlets at δ 1.188 and 0.077 each integrating for one proton were indicative of 7-CH_3 and 8-CH_3 groups respectively.

The ^{13}C-NMR spectrum (deuteropyridine, Figure 4.16) showed signals for 18 carbons. Based on the analyses of the IR, ^{1}H NMR and ^{13}C NMR spectra, the structure (24) has been proposed for the compound LZ4. It is named as (2-hydroxy-di-cyclo 2,5-di-ene 7,8 dimethyl 3-*O*-β-D-glucopyranoside).



(24)

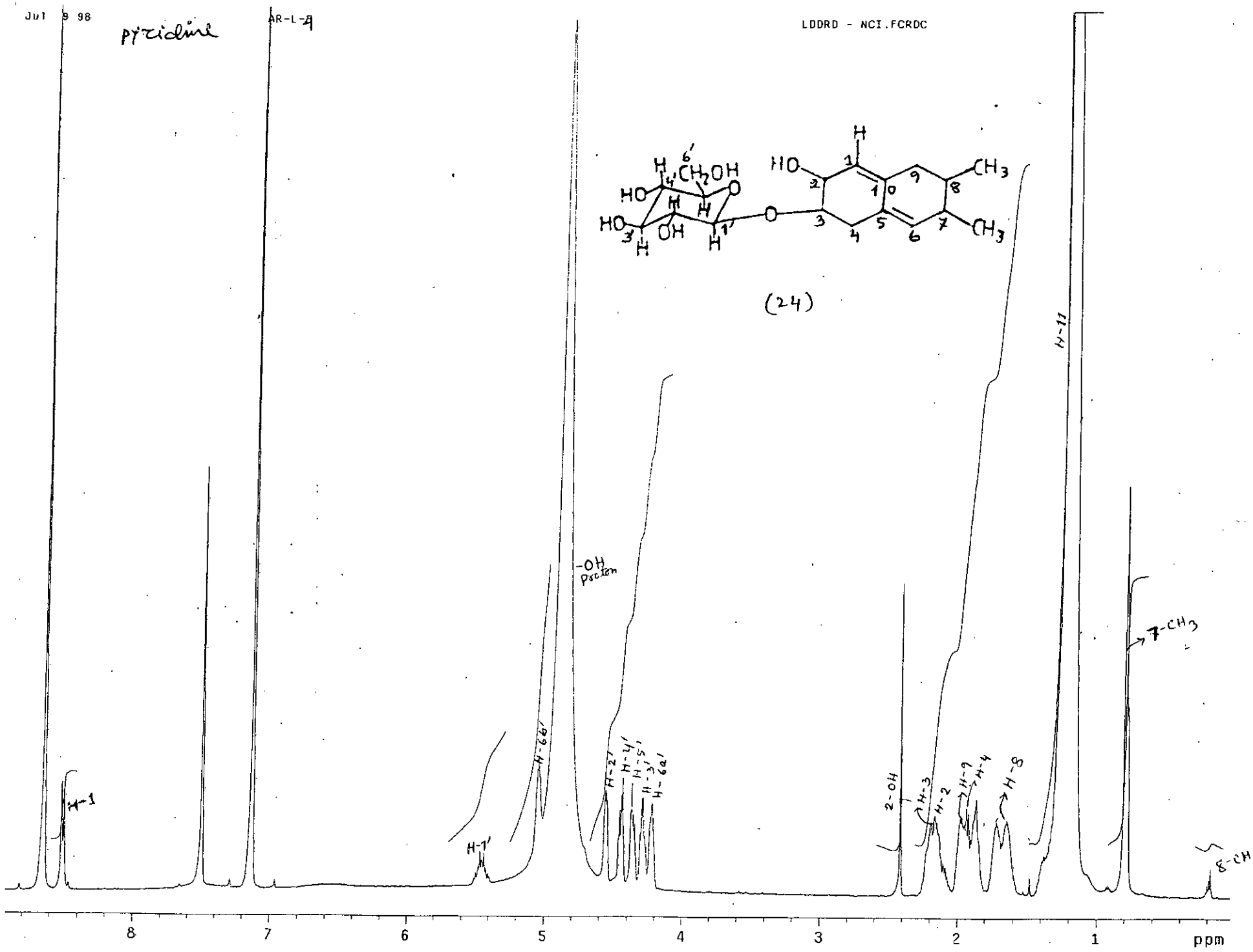


Fig. 4.15 : ^{1}H NMR spectrum of LT4 (24)

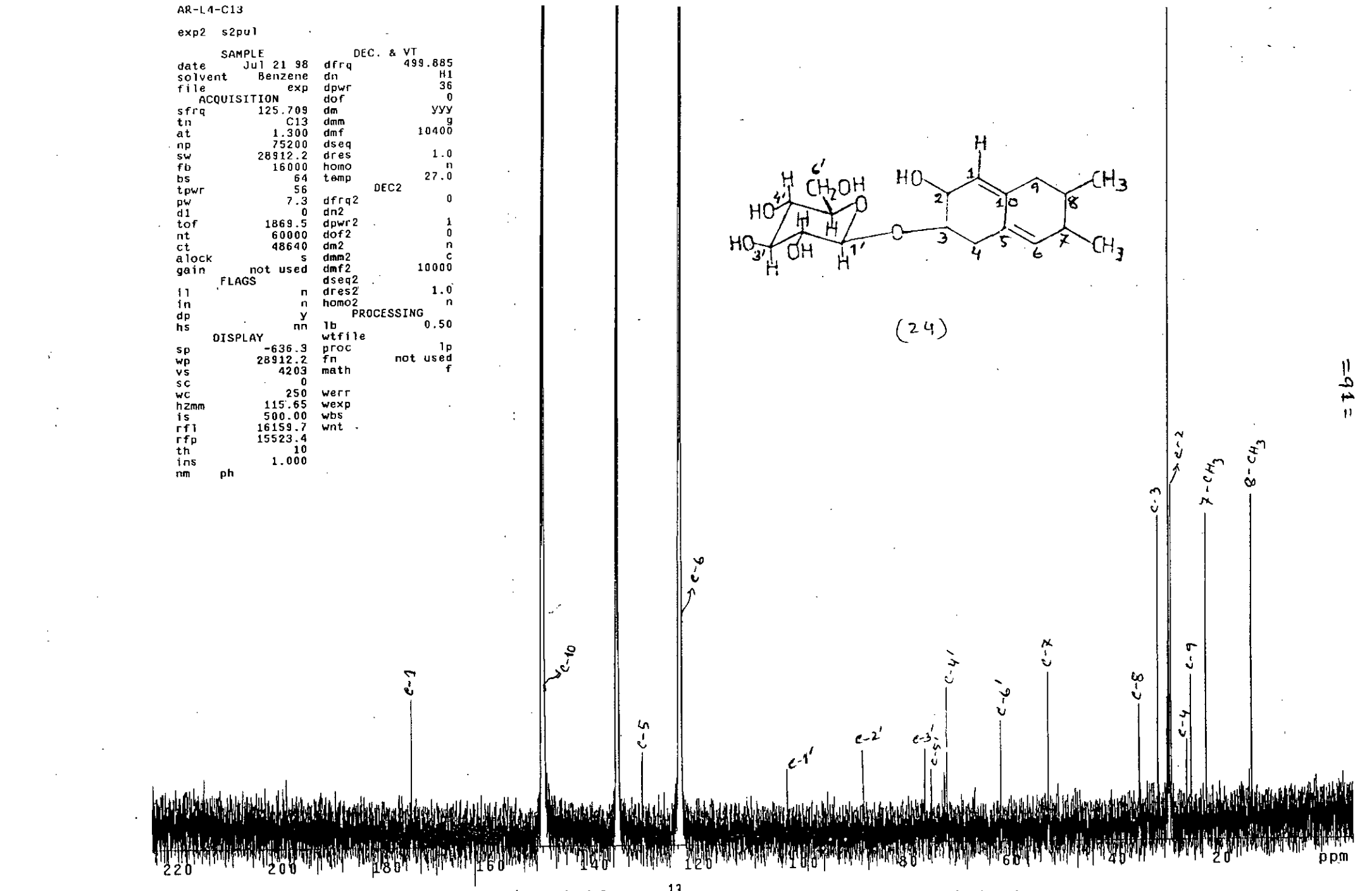


Fig. 4.16 : ^{13}C NMR spectrum of LZ4 (24)

The isolation of a 12 carbon constituted genin glycosidically linked up with a glucose molecule from any other species of the genus Leucas has not been previously reported. However, the occurrence of a good number of glycosides has been reported (ref). Therefore, the isolation and characterization of the compound (24) receives support from the biogenetic ground where $CH_3CO-S-COA$ takes part in the synthesis of organic compounds. Since two carbon units are added in each step, nine $CH_3CO-S-COA$ units have been combined together to give the compound (24). This compound with the proposed structure (24) is expected to be a novel compound.

4.1.1.6 : CHARACTERIZATION OF LZ5

LZ5 was obtained as a silky white needle crystalline substance from the crude MeOH extract of the stems of *Leucas Zeylanica Linn.* by crystallization. It melting point was 353-355^{0}C. It was soluble in water and sparingly soluble in hot methanol.

The compound does not respond to the silver nitrate test for halogen. But on fusion with sodium it responds to the test for halogen giving a light yellow heavy precipitate. Its IR spectrum (Figure 4.17) also showed a band at 820 cm^{-1} indicating the presence of a halogen atom in it (Lambert et al., 1987). It responds vigorously to phenol-sulphuric acid test for sugar. Although its IR spectrum (Figure 4.17) was a representative of an organic compound, it is surprising enough being not able to see any signals either in the ^1H NMR (Figure 4. 18) or ^{13}C NMR spectra (Figure 4.19).The information obtained from IR spectrum suggests that a very tiny amount of an organic compound might have adhered with LZ5.Its major part is now considered as a highly halogenated compound.

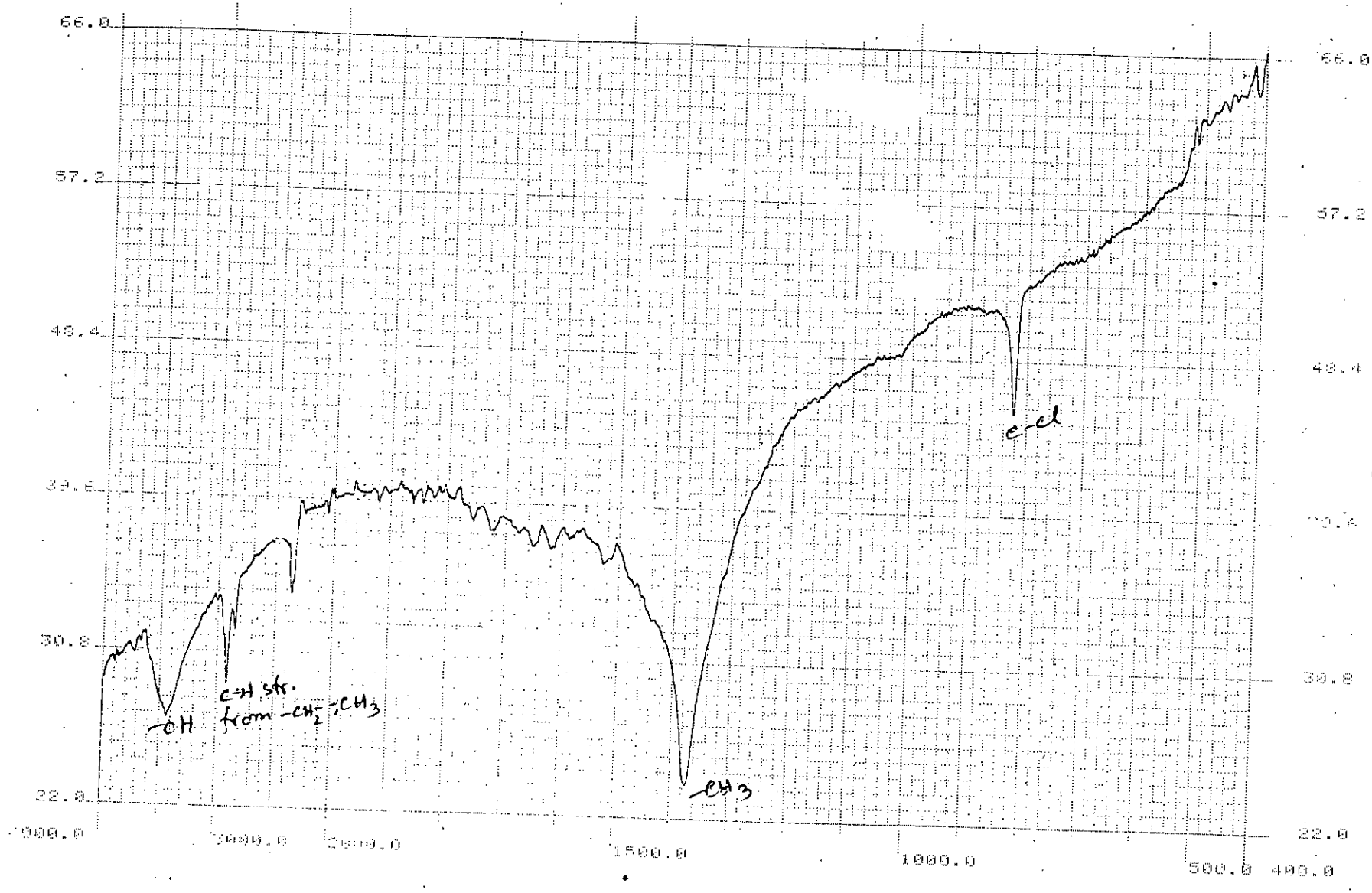


Fig. 4.17 : IR spectrum of LZ5

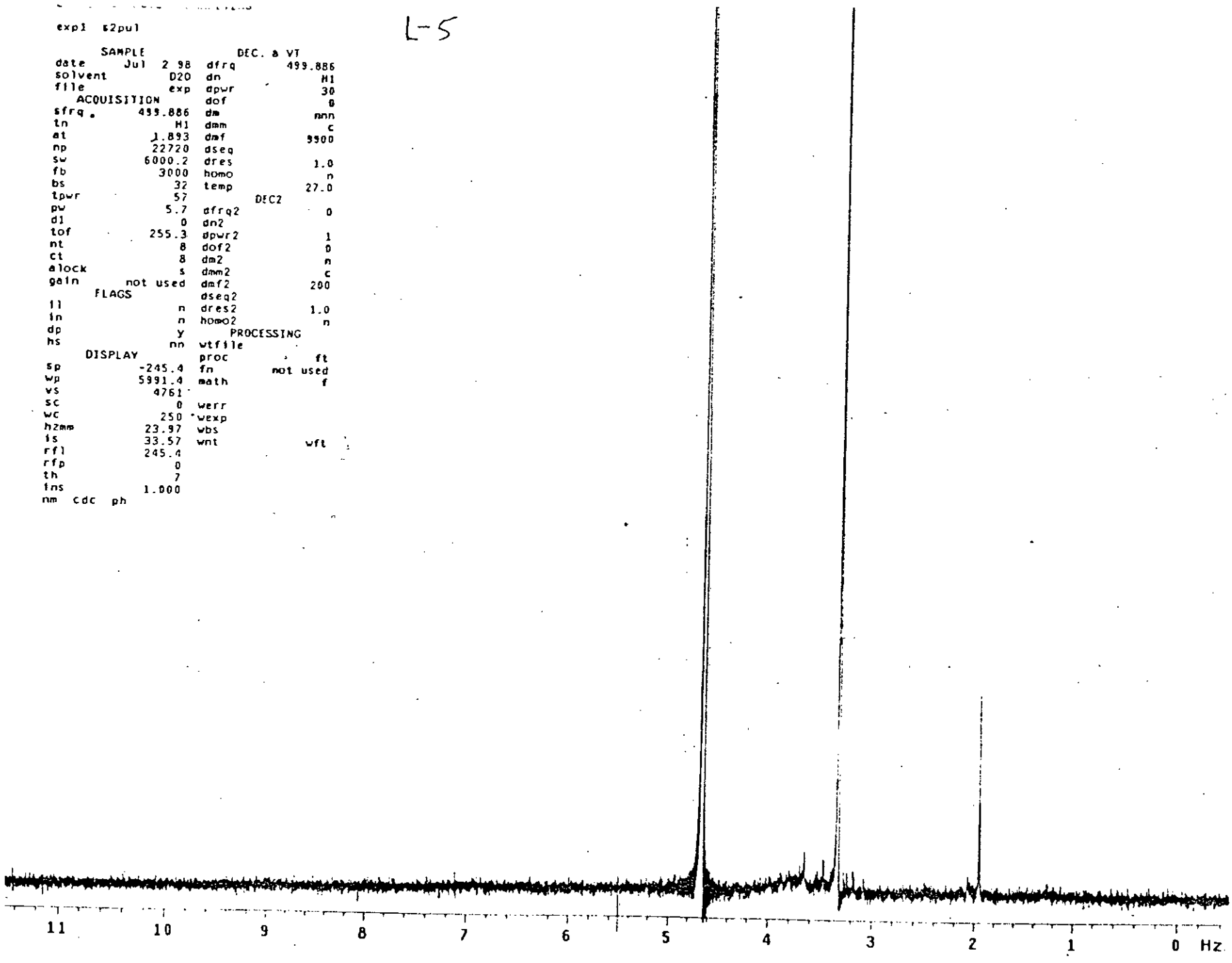


Fig. 4.18 : ^{1}H NMR spectrum of LZ5

exp1 s2pul

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file	exp	dpwr	30
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tn	C13	dmm	g
at	1.300	dmf	9500
np	75200	dseq	
sw	28512.2	dres	1.0
fb	16000	homo	n
bs	64	temp	27.0
tpwr	60	DEC2	
pw	7.3	dfrq2	0
d1	0	dn2	
tof	1869.5	dpwr2	1
nt	4000	dof2	0
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il	n	dres2	1.0
in	n	homo2	n
dp	y	PROCESSING	
hs	nn	lb	0.50
DISPLAY		wtfile	
sp	-644.0	proc	lp
wp	28512.2	fn	not used
vs	5	math	f
sc	0		
wc	250	werr	
hzmm	115.65	wexp	
is	500.00	wbs	
rfl	644.0	wnt	
rfp	0		
th	68		
ins	1.000		
nm	ph		

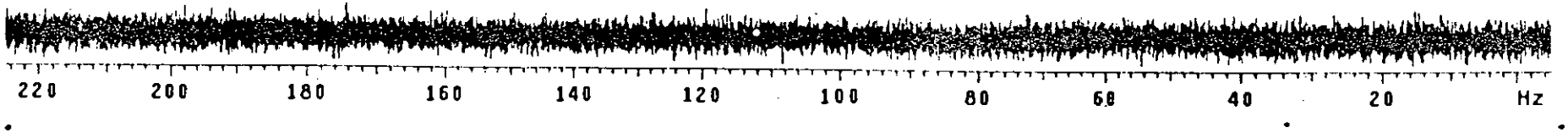

Fig. 4.19 : ^{13}C NMR spectrum of LZ5

4.1.1.7 : ANALYSIS OF THE FRACTION LZW ISOLATED FROM 80 % EtOH EXTRACT :

Paper chromatographic analysis of LZW revealed the presence of glucose, galactose, arabinose and xylose in the fraction LZW. Reduction of the fraction LZW with sodium borohydride, preparation of alditol acetate of the reduced fraction followed by GLC analysis confirmed the presence of glucose, galactose, arabinose and xylose in the fraction LZW.

4.1.2.1 : CHARACTERIZATION OF THE COMPOUND GMP1 :

The compound GMP1 was isolated as a white crystalline substance(150 mg) from the petroleum ether extract of the stems of Gray microcos. It was viewed as a bluish single spot under uv light. It gave a single deep yellowish spot when the tlc plate was stained with iodine vapour in a closed iodine chamber. After removal of the iodine vapour on drying the TLC plate in air, the plate was sprayed with vanillin sulphuric acid spray when a violet spot(R_f value: 0.75, Pet. ether: EtOAc= 3:1) was visualized. It showed a very sharp melting point at 267^{0}C.

The IR spectrum (Figure 4.20) of GMP1 showed a sharp band at 3460 cm-1 indicative of a free hydroxyl group (-OH). The bands at 2900 , 2860 cm^{-1} together with the bands at 920 and 790 cm^{-1} are suggestive of methylene and methyl groups. These also receive support from the bands at 1380 and 1450 cm^{-1}. The sharp band at 1700 cm^{-1} is exhibitive of a carbonyl group. This lower frequency absorption band can be taken as a confirmation of the presence of a carboxylic acid group (Lambert et al., 1987) in it. This

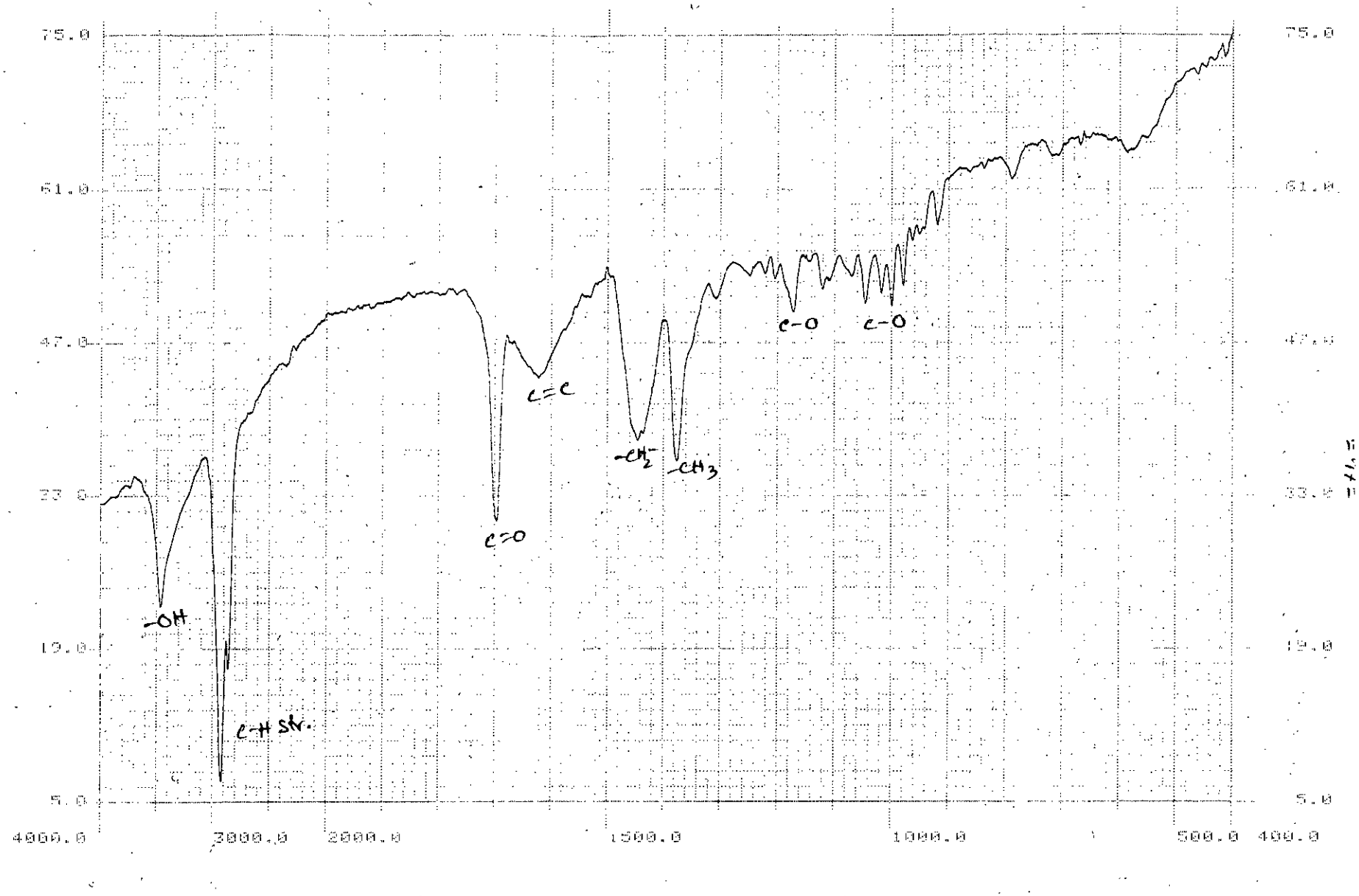

Fig. 4.20 : IR spectrum of the compound GMP1

carbonyl band along with C-O stretching at 1000 cm^{-1}, 1070 cm^{-1}, 1170 cm^{-1} and the hydroxyl band is a typical for a carboxylic acid. The band at 1620 cm^{-1} is indicative of a C=C moiety. The intensity of the band at 1380 cm^{-1} is higher than that of the band at 1450 cm^{-1}. This observation suggests that more CH_3 is present than CH_2.

^1H NMR spectrum (60 MHz, $CDCl_3$, TMS, Figure 4.21) of GMP1 showed a bunches of signals within 0.85 - 1.56 together with tiny signals at 2.6, 3.3, 3.8, 5.26 is a characteristic of a triterpenoid carboxylic acid molecule (Bashir et al., 1982).

4.1.2.2 : CHARACTERIZATION OF GMFA AS A FATTY ACID :

The compound GMFA was isolated as a brownish crystalline substance (200 mg) being wet with an oily layer from the petroleum ether extract of the stems of Gray microcos. It was viewed as a single yellowish spot under UV light. It melted at 71^{0}C.Its IR spectrum (Figure 4.22) showed a comparatively broader and shorter band at 3400 cm^{-1} indicative of a hydroxyl group (-OH).The bands at 2900,2840 cm^{-1} together with the band at 720 cm^{-1} are suggestive of methylene and methyl groups. These also receive support from the bands at 1370 and 1450 cm^{-1}. The sharp band at 1730 cm^{-1} is exhibitive of a carbonyl group. The band at 1450 cm^{-1} is of much more higher intensity compared to the band 1370 cm^{-1}. Furthermore, the IR spectrum showed a band at 1620 cm^{-1} indicative of a C=C functionality which receives strong support from the intensed band at 800 cm^{-1}. This functionality together with the characteristic bands for -OH, C=O and more CH_2 compared to CH_3, suggests its identity as a fatty acid (Lambert et al, 1987).

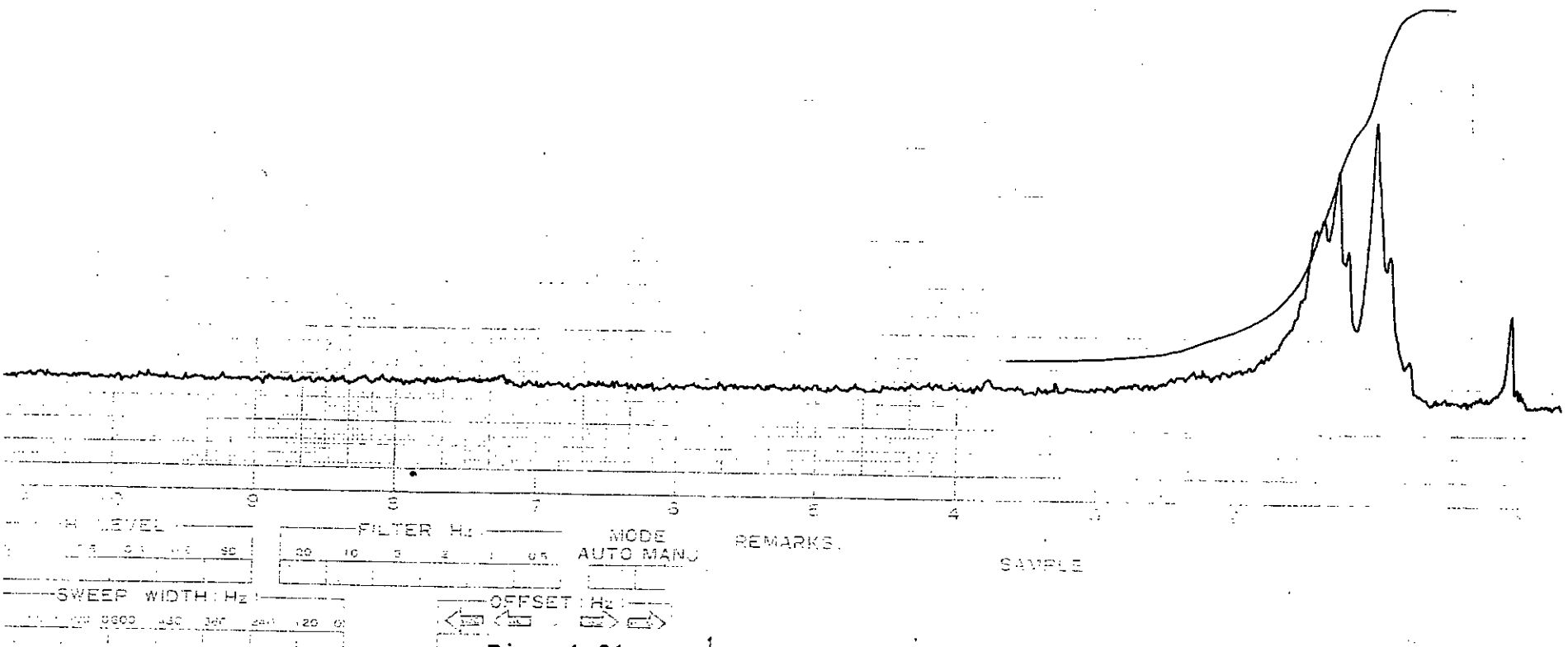

Fig. 4.21 : ^{1}H NMR (60 MHz, $CDCl_3$) of the compound GMP1

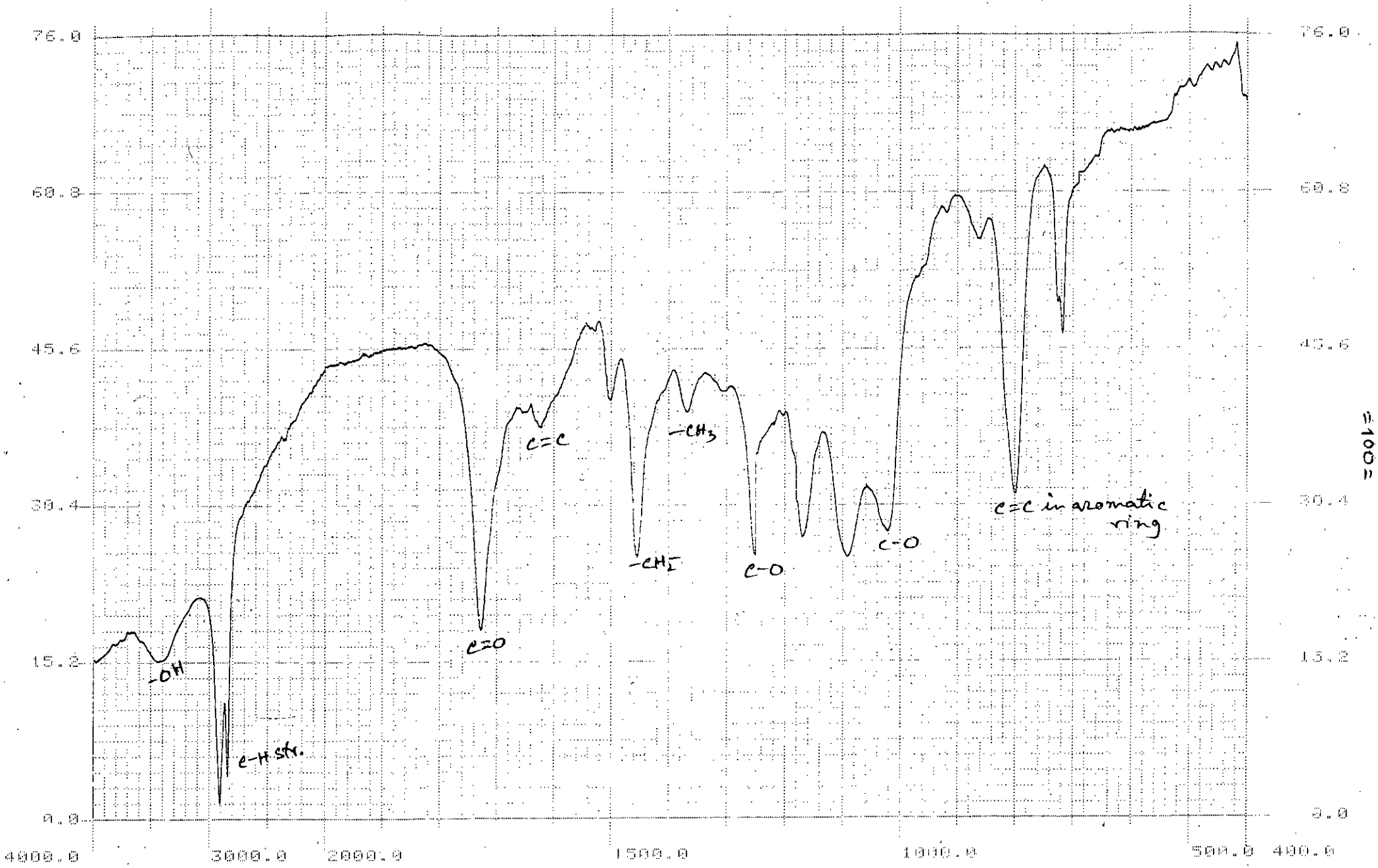

Fig. 4.22 : IR spectrum of the compound GMFA

4.1.2.3 : CHARACTERIZATION OF THE COMPOUND GME2 :

The compound GME2 was isolated as light brownish amorphous substance (25mg) from the ethyl acetate extract of the stems of Gray microcos. It was viewed as a bluish single spot under uv light. It gave a single deep yellowish spot when the tlc plate was stained with iodine vapour in a closed iodine chamber. After removal of the iodine vapour on drying the tlc plate in air, the plate was sprayed with vanillin sulphuric acid spray when a violet spot (R_f value: 0.82, EtOAc: MeOH= 1:1) was visualized. It showed a very sharp melting point at 297^{0}C.

The IR spectrum (Figure 4.23) of GME2 showed a band at 3400 cm^{-1} indicative of a free hydroxyl group (-OH). The bands at 2910, 2860 cm^{-1} together with the bands at 920 and 800 cm^{-1} are suggestive of methylene and methyl groups. These also receive support from the bands at 1360 and 1450 cm^{-1}. The band at 1740 cm^{-1} is exhibitive of a carbonyl group in a cyclic system. This band is comparable to that of either an aldehydic or a ketonic compound. But The absence of the bands at 2750 cm^{-1} and 2850 cm^{-1} confirms that this compound is not aldehydic (**Lambert et al.**, 1987). This carbonyl band along with C-O stretching at 1020 cm^{-1}, 1070 cm^{-1}, 1020 cm^{-1} , the C=C absorption band at 1620 cm^{-1} and the hydroxyl band is a typical for a cyclic ketonic compound. The intensity of the band at 1380 cm^{-1} is closely comparable to that of the band at 1450 cm^{-1}. This observation suggests that CH_3 is present as many as CH_2 . Its IR spectral data were compared with the published data for the compounds reported from the species belonging to the genus *Gray* and were not found in agreement at all. Therefore, this compound is being expected to be a new one.

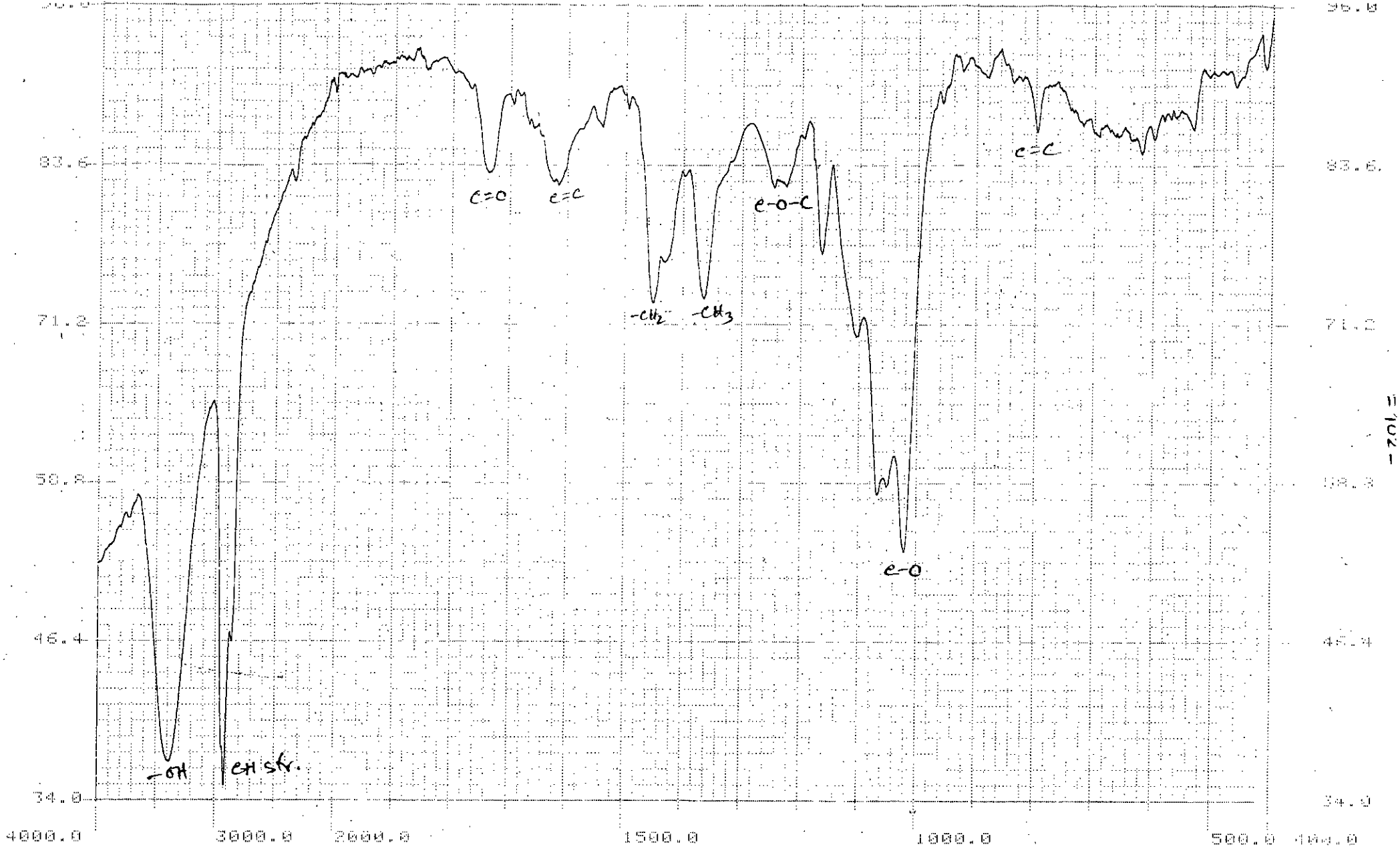

Fig. 4.23 : IR spectrum of the compound GME2

4.1.2.4 : CHARACTERIZATION OF THE COMPOUND GMM3 :

The compound GMM3 was isolated as light pinkish colour amorphous substance (50mg) from the methanol extract of the stems of *Gray microcos Linn.* It was viewed as an orange- yellow spot with Dragendorff's reagent.Its IR spectrum (Figure 4.24) showed a broad band centered at 3400 cm^{-1} indicative of a hydroxyl group (-OH). The band at 1620 cm^{-1} together with the bands at 650 cm^{-1} and C-O str., a broad band at 1100 cm^{-1} is suggestive of an internal salt like compound as it contains nitrogen functionality (bands 600, 1390, 460 cm^{-1}). The occurrence of this alkaloid for the first time in this plant receives support from the report of isolation of an alkaloid from Gray tenax (Garg et al, 1990).

4.1.3.1 : SIGNIFICANCE OF THE COMPOUNDS ISOLATED FROM *LEUCAS ZEYLANICA LINN. AND GREWIA MICROCOS LINN.* :

The compounds (22, 23 and 24) isolated from the plants are of steroidal glycoside ornamented with an epoxy ring .The functionalities such as C=C, epoxy ring, four cyclic ring, a functionalized side chain together with a sugar molecule should have both hydrophobic and hydrophilic properties .Therefore, these compounds are thought to undergo interaction with the receptor molecule in the biological system.This structural knowledge can be taken as an indication to support the present use of this plant for the remedy of ulcer, snake-bite,skin diseases and headache. The report of the isolation of betulin (4),an anticancer agent from *Grewia bicolor* (Jaspers et al., 1986) has attracted the attention of the scientists to pursue more studies on these plants. The

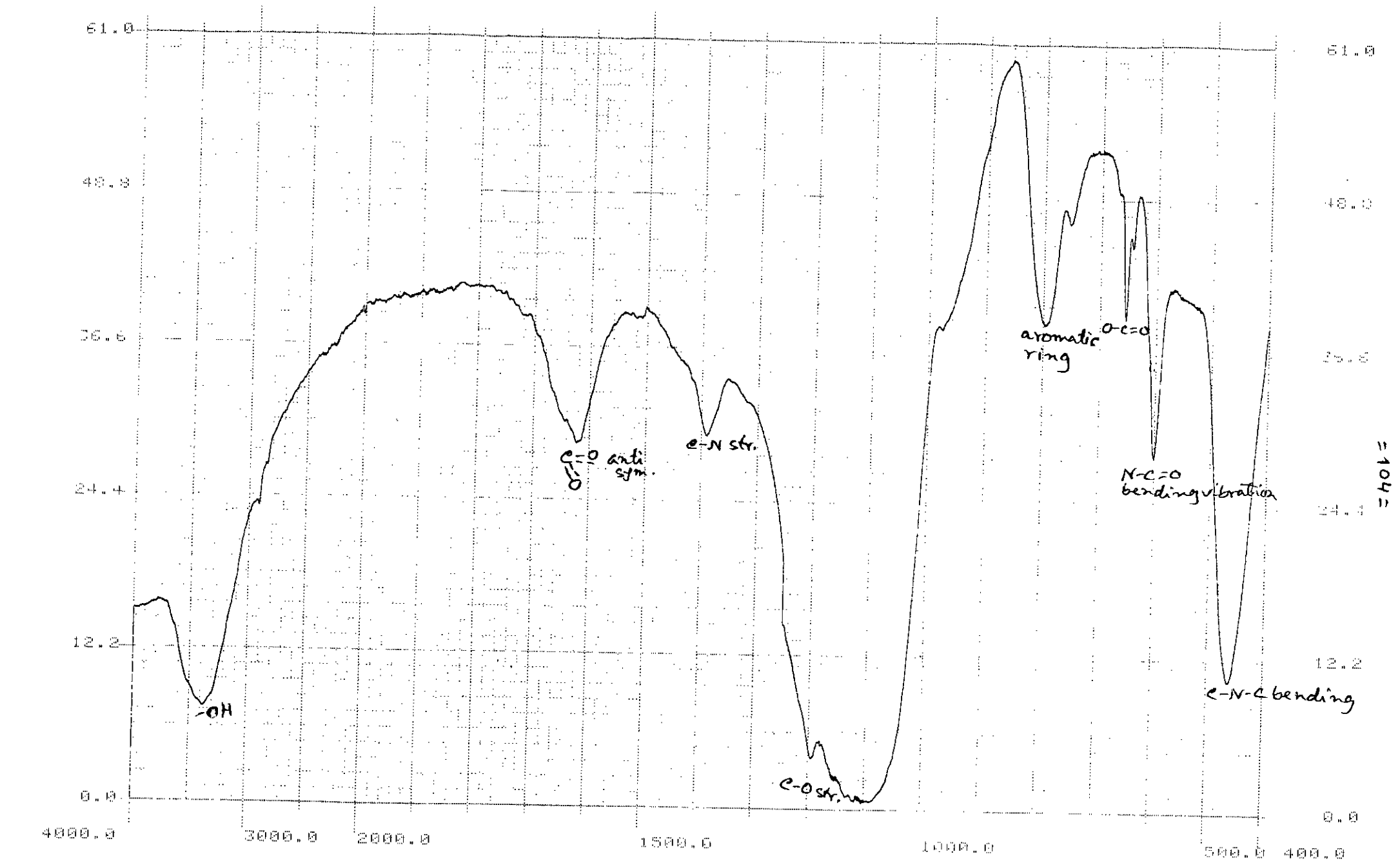

Fig. 4.24 : IR spectrum of the compound GMM3

expected medicinal importance is to be corroborated by biological experimentations.

4.1.4.0 : CHEMOTAXONOMIC SIGNIFICANCE :

The knowledge gained so far from the identification of compounds (1, 2, 3, 4, 11, 12, 13, 14 and 15) in the species of the genera Leucas and *Grewia* suggest that the occurrence of tetracyclic and pentacyclic compounds can be taken as a chemotaxonomic marker because of their common biosynthetic pathway. More work on these plants would enrich this chemotaxonomic significance.

5.0 CHAPTER 5

5.1.0 : REFERENCES :

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