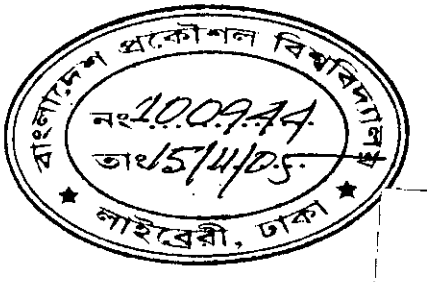
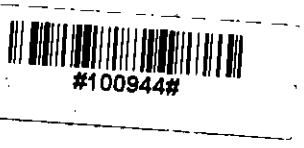
Chemical Studies on the leaves of Anthamul (*Tylophora indica*)

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





SUBMITTED BY
EXAMINATION ROLL NO. 100003101F
(SESSION : OCTOBER-2000)

September, 2005

ORGANIC RESEARCH LABORATORY
DEPARTMENT OF CHEMISTRY
BANGLADESH UNIVERSITY OF ENGINEERING
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THESIS ACCEPTANCE LETTER

We hereby recommend the thesis entitled "Chemical Studies on the Leaves of Anthamul (*Tylophora indica*)" presented by Umme Rayhan, Roll No. 100003101 F, Registration No. 001021, Session October-2000 to accept as partial fulfilment of the requirements for the degree of Master of philosophy (M.Phil.) on 26th September, 2005.

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TO
MY BELOVED PARENTS
AND
FRIENDLY HUSBAND

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, Bangladesh University of Engineeing and Technology (BUET), Dhaka, under the joint supervision of Dr.A.K.M. Matior Rahman, Professor, Department of Chemistry (BUET) and Dr. Md. Abul Hashem, professor, Departement of Chemistry, Jahangirnagar University, Savar, Dhaka during the period starting from October, 2001 to August, 2005. I, further, 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 quotations have been marked by quotation marks.

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Author

SUMMARY

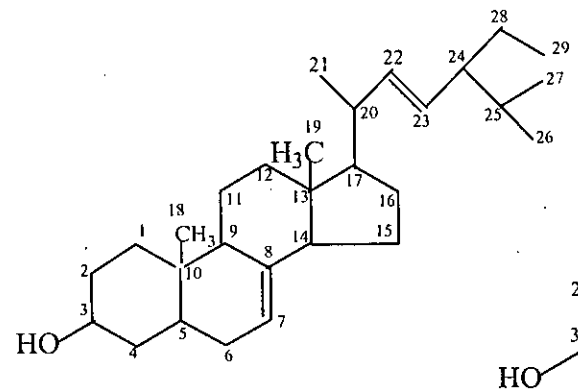
Tylophora indica Syn. *Tylophora asthmatica* of the family **Asclepiadaceae** grow abundantly all over Bangladesh and India. In Bangladesh it is popularly known as "Anthamul". It is an important medicinal plant of our country and is used for the treatment of several diseases specially for the treatment of asthma, dysentery and emesis[12-15].

A review on the phytochemical investigations on the herb revealed that along with other compounds, quite a large number of alkaloidal compounds[20-25] have been isolated from its roots, stems and aerial parts. But in comparison to the work on its stems and roots, little work has been done on its leaves. As root, stem and aerial parts of the plant contained alkaloids, it is quite logical that its leaves should also contain alkaloids amongst other compounds. Alkaloids being physiologically active compounds, our main objective was to isolate, separate and purify alkaloidal compounds from the leaves of anthamul and to determine the molecular architecture of the isolated alkaloids along with other compounds isolated.

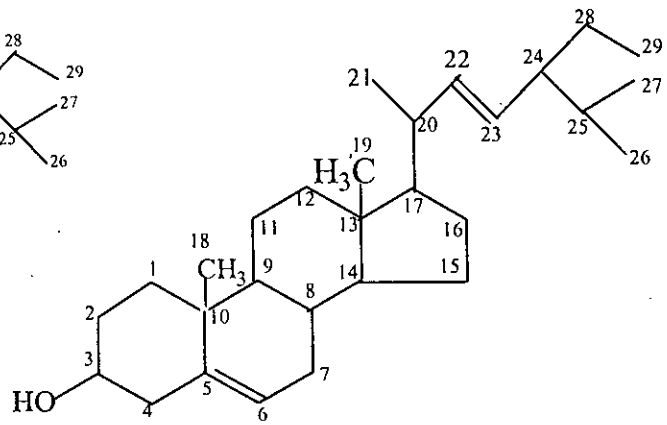
The herb *T.indica* was cultivated in the Club premises of the Bangladesh University of engineering and Technology (BUET). The leaves were collected, dried in the shade and grinded into powder for the purpose of the experiment. The powder obtained was successively extracted with Pet. ether, EtOAc and MeOH.

Separation and fractionation followed by chromatographic analyses of the EtOAc extract R_{EA} resulted into the isolation of the three pure compounds EA-2 EA-3 and EA-4. Fractionation followed by purification of the various extracts enable us to isolate the pure compound A_5 from methanol extract, ME. All the four compounds responded to the usual color reactions of alkaloids showing that they are alkaloidal in nature.

On the basis of IR, ^{1}H-nmr, ^{13}C-nmr spectral analyses, the molecular formula of the compound EA-2 was found as $C_{29}H_{48}O$. ^{13}C-nmr spectral data of the compound EA-2 was in full agreement with the published data of the stigmasterol[31].Thus on the basis of the chemical and spectral analyses, tentatively the following structure (20) may be assigned for the compound EA-2 and it can be named as Stigmastan-5,22-di-ene-3β-ol in comparison with the established structure (19) of the compound Stigmastan-7,22-di-ene-3β-ol. Thus the compound EA-2 is a sterol.

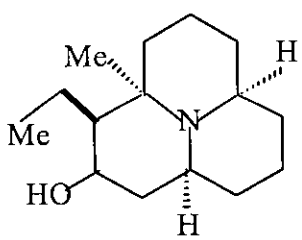


Stigmastan-7,22-di-ene-3β-ol (19)

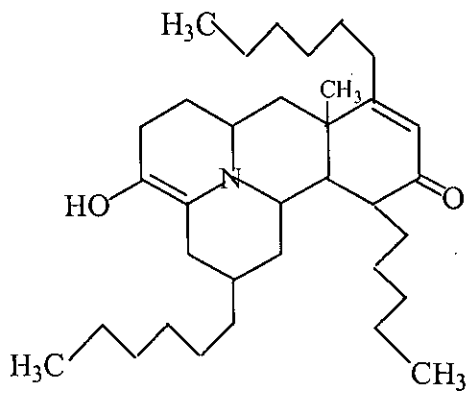


Stigmastan-5,22-di-ene-3β-ol (20)

On the basis of IR, ^{1}H-nmr, ^{13}C-nmr spectral analyses, the molecular formula EA-3 was found as $C_{34}H_{57}NO_2$. The molecular mass of the compound on the basis of its Molecular formula $C_{34}H_{57}NO_2$ is 511. Although there is no molecular ion peak in the mass spectrum, the base peak found in the mass spectrum at m/z 284 can be easily obtained by theoretical calculation followed by the mass fragmentation shown in the section 4.1.5. Thus on the basis of all the chemical and spectral analyses, the following tentative structure (22) may be assigned for the compound EA-3 which is a derivative of the alkaloid Porathericine with the established structure (21).



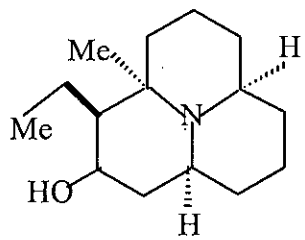
(21)
Porathericin



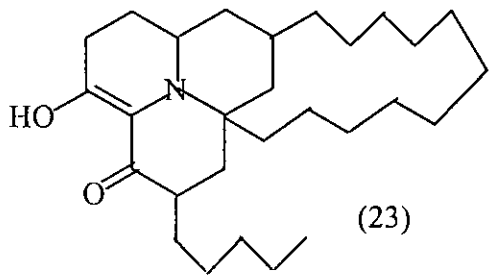
(22)
Compound EA-3

On the basis of IR, ^{1}H-nmr, ^{13}C-nmr spectral analyses, the molecular formula of the compound EA-4 was found as $C_{28}H_{47}NO_2$. The molecular mass 429 calculated on the basis of this molecular formula is supported by the mass spectrum showhing a molecular ion peak at m/z 429 followed by the fragmentation pattern as shown in the section 4.1.6. on the basis of all the chemical and spectral analyses, tentatively the following structure

(23) may be assigned for the compound EA-4 which is also a derivative of the alkaloid Porathericine with the established structure (21).

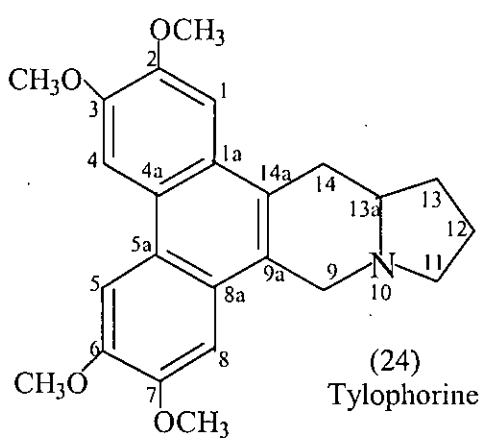


(21)
Porathericin

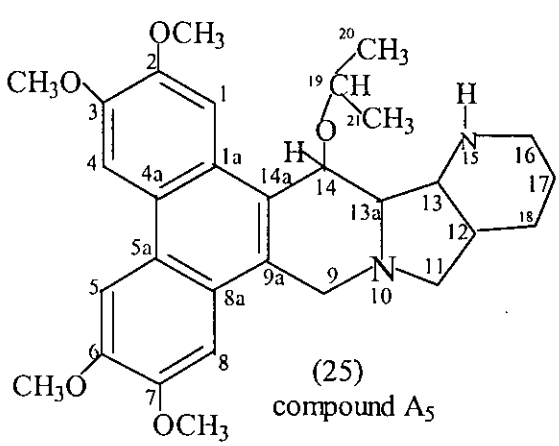


(23)
Compound EA-4

On the basis of IR, ^{1}H-nmr, ^{13}C-nmr spectral analyses, the molecular formula A_5 was found as $C_{30}H_{38}N_2O_5$. Thus with 38 protons from ^{1}H-nmr spectrum, 30 carbons from ^{13}C-nmr and ^{13}C - Dept spectra along with the functional groups from IR spectrum, the molecular formula of the compound A_5 can be written as $C_{30}H_{38}N_2O_5$ .Though the mass spectra of the compound is not available, considering its available ^{1}H-^{1}H COSY and ^{1}H -^{13}C COSY(HMBC), the following structure (25) may be tentatively assigned for the compound A_5 with the molecular formula $C_{30}H_{38}N_2O_5$. This compound A_5 with the designed structure (25) is a derivative of the alkaloid **Tylophorine**[24] with the established structure(24).



(24)
Tylophorine



(25)
compound A_5

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1.1 : General Introduction :

Three basic needs of mankind are food, cloth and dwelling for which they are solely dependant on the plant kingdom. Apart from these three basic needs, at the dawn of civilization, for want of any synthetic drug people also had to use plants and their extracts to combat decay, disease and death. As time went on, development of Science and Technology rewarded mankind with the discovery of the modern drugs and pharmaceuticals. The knowledge of Chemistry and Pharmacy during the beginning of nineteenth century played a very important role in the study of natural products leading to the drug discovery from medicinal plants and herbs. Morphine, the hypnotic and anaesthetic principle isolated from opium; quinine the anti-malarial drug from chincona bark and cocaine isolated from coca leaves used as local anaesthetic are some examples in the list of early discovered drugs. It was followed by the discovery of reserprine, an alkaloid from *Rauwolfia serpentina*. After the discovery of the alkaloids of the Rauwolfia group used in the mental conditions including tension and anxiety as well as in the treatment of hypertension more and more potent drugs continued to be isolated from plant bodies. The alkaloids vincristine and vinblastin isolated from *Vinca rosea Linn* are the potential drugs used against blood cancer, leukemia. The tincture made from *Ephedra Vulgaris* is effective in the treatment of asthma, cardiac failure etc. Every part of the plant *Azadhirachta indica* (Beng. Neem) is reported to have medicinal properties.

Though with the advent of modern synthetic drugs the use of traditional herbal medicines declined sharply all over the world during the period of 1980[s], the pendulum is now swinging back and the use of herbal medicine is again gaining increasing attention round the globe. As an example, during the past twenty five years, "kampo", the Japanese traditional medicine made an impressive come back because 70% of 200,000 Japanese physicians have been regularly prescribing "kampo" drugs for all sorts of ailments ranging from gynecological disorder to cardiovascular diseases. Even the National

Institute of Health, USA has given a new thought as an alternative to synthetic drugs, e.g. ginkgo biloda to prevent dementia, glucosamine chondroitin sulphate for arthritis, shark cartilage for lung cancer and Gonzalez protocol for pancreatic cancer[1]. A survey of the registered Swedish Drugs in the early seventies of the 20th century has shown that natural product account for 51% of all medicinal preparation[2] and this might be true for many other countries of the world. Sticher estimated that 50% of all drugs in industrialized countries are natural products[3]. In many countries of the world native medicinal plants are thus looked upon as the possible additions to the WHO list of "essential drugs" once their medicinal value is clinically established[4].

Therefore, at the beginning of 21st century, there has been a tremendous resurgence of public interest in the study and use of traditional herbal medicines and many developing countries like ours have decided to pay serious attention to explore the possible utilization of herbal medicine in primary health care. It is worthwhile to mention here that the tropical weather and fertile soil of Bangladesh has made it an ideal place for the growth of a diverse medicinal plants and herbs. With such a rich heritage of medicinal plants Bangladesh is regarded as a storehouse of herbal medicines in the South East Asia.

As the modern medical science is getting more and more advanced, the allopathic treatment is getting more and more costly and going out of the reach of our common people. On the contrary, the traditional medicines though less reliable yet free of any side effect. These are much more cheaper and readily available for the poor people of this country. From time immemorial, these herbal medicines isolated from various plants and herbs took primary care of health for thousands and millions of people of this country as they were used as remedy for multiple diseases under various traditional systems e.g. the Ayurvedic, the Unani and the Kaviraji. But for various reasons only a very few of these plants have been investigated chemically and yet then not systematically. The truth is that most of them have not yet undergone any phytochemical and biomedical investigations. As a result now-a-days Bangladesh at the cost of a lot of foreign currency imports huge quantities of plant materials and their extracts which are used primarily for the

manufacture of the Ayurvedic, the Unani, the Kaviraji and the Homoeopathic medicines and also as basic raw materials in the pharmaceutical and agrochemical based industries. Thus it is the high time for us to pay serious attention to the phytochemical, pharmacological and clinical evaluations of our medicinal plants and herbs that will lead to the discovery of newer and newer drugs to combat complicated diseases e.g. heart diseases, cancers, diabetes, rheumatism, arthritis and AIDS.

1.2 :Medicinally important plants and herbs of our country :

The following is a brief list of the important medicinal plants and herbs that are traditionally used as remedies against various ailments by the Ayurvedic, Unani and Kabiraji physicians of our country.

Abortion:

Leaves and seeds of *Achyranthes aspara* L.(Beng. Apang) (Fam. Amaranthaceae), Rhizomes of *Gloriosa Superba* L. (Beng. Ulatchandal) (Fam. Liliaceae) are used as folklore medicines to prevent abortion by our local people [5].

Anaemia:

The flowers of *Gmelina arborea* (Beng. Gamari), the fruits of *phyllanthus emblica*[6] (Beng. Amla, Amlaki) *Piper Nigram* [6](Beng. Golmorich), the leaf and stem of fresh green plants of *Scoparia dulcis*[6] (Beng. Madhumisti), plant juice of *Oxalis corniculata* [6] (Beng. Amrul, Amboli), the bark of *Ixora arborea* [6] (Beng. sweet Rangan), the plant *Glycosmis Pentaphyla* [6] (Beng. Matkila Datmajan), the leaves of *Hygrohlila auriculata* [6] (Beng. Kulekeshara, Talmakhna) the roots of *Ipomea turpetlum* [7](Beng. Dud kalmi) the seeds of *Trigonella toenumgralicum* [8](Beng. Methi), the barks of *Acacia catechu* [9](Beng. khoyer) *Terminalia arjuna* [9](Beng. Arjun, Aurjuna) the fruits of *Coccinia indica* [9] (Telakucha), *Terminalia chebula* [9](Horitoki), the plants *Eclipta alba* [9](Beng Keysuria), *Hydrocotyl asiatica* [9](Beng. Thankuni), etc. are used as medicine for the treatment of anemia by the rural people of our country.

Antidiabetes:

The roots and leaves of *Coccinia indica* (Beng. Telakucha), are reported to have sugar lowering activity and clinical tests on the capsule made of it have proved to be so [9]. The leaves *of Jatrupa curcas L.* (Beng. Jamalgota, Baghverenda)[10] , the seeds of *Mangifera indica* [9] *Linn.* (Beng. Aam), the leaves of *Michelia champace* [9] *Linn* (Beng. Champa, Chompa), the leaves and seeds of *Musa Sapientum* [9] (Beng. Kola), the barks of *Eugenia jambos*[9] (Beng. Golap jam) *Momordica charantia* [11](Beng. Karulla, Usta), the seeds of *Trigonella foenumgraecum* [8](Beng. Methi) the leaves and flowers of *Vinca roea* (Beng. Nayntara) (Fam. Apocynaceae), roots of *Asparagus race-mousus L.* (Beng. Shatamuli) (Fam. Liliaceae) leaves and seeds of *Sesbania grandiflora* (L.) pers. (Beng. Bak-phul) (Fam. Legummisosae)[5]. are used for the treatment of diabetes.

Antifertility:

The plants like *Cascuta reflexa* [10] (Beng. Tarulata), *Aeacia catechu* [10] (Beng. Khoyer), *Abrus precatories* [6] Linn (Beng. Kunch) *Areca Calechu Linn* (Beng. Supari) *Carica papaya* [6] (Beng. Papay) the roots and leaves of *Piper betel L.*[6] (Beng. Pan, Tambuli), *Plumbago Zeylanica* [6] *L.* (Beng. Cheta, chitra), the stem barks of *Acacia arabica*[6] (Beng. Babla) etc. are reported to have anti-fertility activity. "Shanti bori " a traditional contraceptive pill comprising of a mixture of exudate of *Acecia Catechu* (Beng. Khair), powder seeds of *Tragia involucrata* (Beng. Bichuli), powdered barks of *Acacia arabica* (Beng. Babla) has been shown to inhibit fertility of female rats to about 87.5% without affecting the oestrous cycles of the rats [9]. The roots leaves and flowers of *Hibiscus rosa-sinensis* L. (Beng. Jaba) (Fam. Malvaceae) [5]. are also found to inhibit anti-fertility activity.

Anticeptic:

Most of the people of Bangladesh use various plants as antiseptic for cuts and wounds. The leaves of *Cynodon dactylon* [6] (Beng. Durba, Dubla, Durba gash), bulbs of *Allium Sativum* [6] (Beng. Rasun). leaves of *Pistia stratiotes* [6] L. (Beng. Topapana), seeds of *Cleome viscosa* [6] L (Beng. Hurhuria) (Fam. Capparidaceae) *Oxalis corniculate* [6] L. (Beng. Amrul) *Saccharum officinarum* [6] L (Beng. Akh), *Azadirachta indica* [7] (Beng. Neem), *Eucalyptus globules* [9], *Trigonella foenumgraecum* [10] (Beng. Methi), leaves of *Tridax procumbens* L. (Beng. Tridhara) (Fam. Compositae), upper Part of the plant of Eupatorium triplinerve vahl. (Beng. Ayapan) (Fam. compositae) whole plant of *Cymbopogon citrates* (DC) stapt. (Beng. Lemon grass) (Fam. Gramineae) [5] etc. are used by the people against antiseptic for cuts and wounds.

Appetiser:

Leaves and roots of *Scoparia dulsis L* (Beng. Bandhonia) (Fam. Scrophulariaceae), roots of *Asparagus racemosus L.* (Beng. Shatamuli) (Fam. Liliaceae); leaves of *Mohania macrophylla* willd (Beng. Moghania) (Fam. leguminosae); fruits of *Phyllanthus emblica* L. (Beng. Amlaki) (Fam. Euphorbiaceae) and fruits of *Terminalia chebula* [5] tetz (Beng. Haritoki) (Fam. Combretaceae) are traditionally used as appetiser by the rural people of Bangladesh.

Asthma:

The fruits of *Mimusops elengi* [9] (Beng. Bakul), *Terminalia chebula* [9] (Beng. Horitoki), the juice of the plant of *Coccinia indica* [6] (Beng. Talakucha) (Fam. Cucurbitaceae), the banks of *Alstonia Scholaries* [9] (Beng. Chatim), *Caeselphinia cristia* [9] (Beng. Nata, Nata Koromza), *Euzenia jambolana*[9] (Beng. Jam, Kala jam), *Eujenia jambos*_[9] (Beng. Golap jam), the leaves of *Datura metal* [9] Linn (Beng. Dhutura, Dhutara) (Fam. Solanaceae), *Ricinus communis*[9] Linn (Beng. Varenda) the barks, fruits and leaves of *Aegel marmelos* [9] (Beng. Bel) *Mangifera indica* (Beng. Aam), the fruit and leaves of *Adhatoda Vasica* [9]

(Beng. Bashok), the barks and leaves of *Calotropis gigantea* [9] (Beng. Akondo) (Fam. Asclepiadaceae, the plants of *Hydrocotyl asistica* [9] Linn (Beng. Thankuni) (Fam. Umbelliferae); roots, leaves, barks and seeds of *Cassis occidentalis L.* (Beng. Bara Kalkaesunde) (Fam. Leguminosae), whole plant of *Cissus quadrangularis L.* (Beng. Harjora) (Fam. Vitaceae); roots, fruits, barks and latex of *Ficus hispida L.* (Beng. Jagadumur) (Fam. Moraceae)[5], whole plant of *Euphorbia hirta L.* (Beng. Dudhi)[5] (Fam. Euphorbiaceae) Fruits and barks of *Terminalia beleria* [5] *Roxb.* (Beng. Bohera) (Fam. Combretaceae)[5]; fruits and seeds of *Elettaria Cardamomum Maton* (Beng. Elache) [5](Fam. Zingiberaceae) root, leaves, flowers and young stems of *Acalypha indica* [5] L (Beng. Muktajhuri) (Fam. Euphorbiaceae), roots and whole plants of *Boerhaavia diffusa*[5] L. (Beng. Punanaba) (Fam. Nyctaginaceae), whole plant of *Marsilea quadrifolica*[5] *L.* (Beng. Sushuni) (Fam. Morsiliaceae), roots, leaves and flowers of *Adhatada Vasica* [5] Nees (Beng. Basak) (Fam. Acanthaceae), leaves and fruits of *Passiflora edulii* [5] L. (Beng. Jhumkalata) (Fam. Passifloraceae), leaves and rhizomes of *Typhonium trilobatum* [5] L. Scott. (Beng. Ghetu Kachu) (Fam. Araceae); roots and fruits of *Solanum Sesembifolium*[5] L. (Beng. Kantakini) (Fam. Solanaceae) and whole plant of *Marselae quadrifolia* L. (Beng. Sushuni) (Fam. Marsiliaceae)[5] have got wider applications as medicine for the treatment of asthma.

Blood Pressure:

The leaves and roots of *Catharanthus roseus* (L) G. Don. (Beng. Nayantara) (Fam. Apocyanaceae), roots of *Rauwolfia Serpentina* Benth. (Beng. Sarpagandha) (Fam. Appocyanaceae) whole plant of *Marselea quadrifolia* L. (Beng. Sushni) (Fam. Marsiliaceae)[5] are used for alleviating blood pressure.

Blood Purifier:

The flowers, leaves and roots of *Tagetes erecta L.* (Beng. Ganda) (Fam. Compositae); roots, leaves and stems of *Amaranthus spinosus* L. (Beng. Kanta notey) (Fam. Amaranthaceae) rhizomes of *Kaemp feria rotunda L.* (Beng. Bhuin Champa), (Fam.

Zingiberaceae) roots and leaves of *Crystolepis buchanani* Roem & Schult (Beng. Dudhilata) (Fam. Apocyanaceae) whole plants of *Enhydra fluctuans* Lour (Beng. Helencha) (Fam. Compositae), rhizomes of *Curcuma longa* L. (Beng. Halud) (Fam. Leguminosae)[5] are used as blood purifier.

Bronchitis:

Fruits, roots, barks and latex of *Ficus hispida L.* (Beng. Jagadumur) (Fam. Moraceae), roots, leaves, barks and seeds of *Cassia occidentalis* L. (Beng. Bara Kalkaesunde) (Fami, Leguminosae), whole plant of *Euphorbia hirta L.* (Beng. Dudhi) (Fam. Euphorbiaceae), roots, seeds, barks and leaves of *Clome viscosa L.* (Beng. Hurharia) (Fam. Capparidaceae), leaves and seeds of *Achyranthes aspera* L. (Beng. Apang) (Fam. Amaranthaceae), whole plant of *Cymbopogon citrates* (DC.) stap f. (Beng. Lemon grass) (Fam. Gramineae), leaves and rhizomes of *Typhonium trilobatum* (L.) Scott. (Beng. Ghelu Kachu), (Fam. Araceae), roots, leaves and stems of *Amaranthus spinosus L.* (Beng. Kata notey) (Fam. Amaranthaceae); rhizomes of *Acorus Calamus L.* (Beng. Boch) (Fam. Araceae); roots and leaves of *Indigoera tinctoria L.* (Beng. Nil) (Fam. Leguminosae); fruits and seeds of *Capsicum frutescens* L. (Beng. Marich) (Fam. Solanaceae); roots, leaves of *Tridax procumbens* L. (Beng. Tridhara) (Fam. Compositae); leaves and flower of *Tagetes erecta* L. (Beng. Ganda) (Fam. Compositae); leaves of *Desmodium gangeticum* L. DC (Beng. Salpani) (Fam. Leguminose) and the whole plant of *Enhydra fluctuans*_Lour (Beng. Helencha) (Fam. compositae)[5] , the leaves and barks of *Acacia aradica* [9] (Beng. Babla); the barks and seeds of *Punica granantum*_[9] (Beng. Dalimgach), the leaves of *Psidium guyava* [9] (Beng. Piyara, Peyara); the leaves and roots of *Lowsonia inermis* (Beng. Mehidi, Mendi) [9] are used by the rural people for the treatment of the bronchitis.

Cancer:

The alkaloids, Vincristin and Vinblastine isolated from *Vinca rosa* [6](Beng. Nayantara) (Fam. Apocynaceae) are being used against blood cancer, leukemia. The latex of *Ficus*

racemora [6] Linn. (Beng Jagadumur) (Fam. Moraceae) is useful as anticancer al against. The leaves of *Rhinacantus nasuta*[6] (Beng. Jaipana) are applied in the treatment of cancer. The plant *Vitex trifolia* [6] (Beng. Panisamula) shows anticancer activity. The plants, roots and fruits of *Xanthium strumarium* [9] Linn. (Beng. Bonokra) is used for the treatment of cancer. The roots of *Asparagus racemousus* L. (Beng. Shathamuli) (Fam. Liliaceae), roots and leaves of *Piper betel L.*(Beng. Pan) (Fam. Piperaceae); seeds and whole plant of *Hyptis Suaveolens poir* (Beng. Tukma) (Fam. Labiatae) and the whole plant of *Xanthium indicum Koen.* ex Roxb. (Beng. Ghagra) (Fam. Compositae) are used against cancer [5].

Constipation:

Leaves, roots and seeds of *Clitoria termatae* L. (Beng. Aporajita) (Fam. Leguminasae); roots and whole plant of *Boerhaavia Diffusa* L. (Beng. Purnanaba) (Fam. Nyctaginaceae) roots, leaves, flower and young stems of *Acalypha indica* L. (Beng. Mukta Jhuri) (Fam. Euphorbiaceae), fruits and seeds of *Eleltaria cardamomum* Maton. (Beng. Elache) (Fam. Zingiberaceae), roots, leaves flowers barks and whole latex of *Calotropis Procera Br.* (Beng Akanda) (Fam. Asclepiadaceae), fruits and barks of *Terminalia belerica* Roxb (Beng. Bohera) (Fam. Combretaceae) whole plant of *Eclipta alba* (L.) Hassk. (Beng. Kesuti) (Fam. compositae) and the leaves of *Aloe indica willd.* (Beng. Grithakumari) (Fam. Liliaceae)[5] are used as folklore medicine against constipation.

Diarrhea :

The capsules made from dried leaves of *Hydrocotile asiatica* [9] (Beng. Thankuni) (Fam. Umbelleferae), *Peoderia foelida.* Oxalis *corniculata* (Beng. Amrul) and *Aegle mormelos* [15] (Beng. Bel) has been found to be clinically effications against diarrhoea. The bark of *Alstonia Scholaris* [6] Linn (Beng. Chatim) is a valuable remedy in chronic diarrhoea. The bark and seeds of *Albizzia lebbech*[6] Linn are given in diarrhea. The roots of *Bergenia ligulate*[9](Beng. Pathorkuchi), *Ipomoea batatas* [6](Beng. Misti alu), the plants of *Cynodon dactylon* [6] (Beng. Durba), roots of *Asparagus racemousus* L. (Beng. Shatamuli) (Fam. Liliaceae), fruits and barks of *Terminalia belerica* Roxb (Beng. Bohera) (Fam.

Combretaceae), leaves of *Paederia foetida* L. (Beng. Gandhabhadal) (Fam. Rubiaceae), roots and leaves of *Phyllanthus amarus* Wab (Beng. Bhui amla) (Fam. Euphorbiaceae) flowers and fruits of *Bombax ceiba* L. (Beng. Shimul) (Fam. Bombaceae) tender leaves of *Lippia alba* L. (Beng. Matkila) (Fam. Verbenceae), whole plant of *Euphorbia hirta* L. (Beng. Dudhi) (Fam. Euphorbiaceae) leaves of *Jasminum Sambac* L. Ait (Beng. Beli) (Fam. Oleaceae), leaves and seeds of *Sesbania grandifflora* L. Pers. (Beng. Bak-Phul) (Fam. Legumminosae), roots leaves and barks of *Phyllanthus reticulates* Poir (Beng. Chitki) (Fam. Euphorbiaceae)[5] etc. are traditionally reported to be effective against diarrhoea.

Dysentery:

The barks and seeds of *Acacia catechu* [9] (Beng. Khoyer) *Aegel marmelos* [9] (Beng. Bel) *Diospyros embryopteris* (Beng. Gub), *Mangifera Indica* [9] Linn.(Beng. Aam), *Phyllanthus embliea* [9] Linn. (Beng. Amloki) the plants and leaves of *Oxalis corniculata* [6] Linn (Beng. Amrul) *Andrographis Paniculata* [9] (Beng. Kalomegh) roots of *Asparagus racemousus L.* (Beng. Shatamuli) (Fam. Liliaceae), fruits and barks of *Therminalia belerica* Roxb (Beng. Bhohera) (Fam. Combretaceae) fruits and barks of *Terminalia chebula Tetz* (Bneg. Harithaki) (Fam. Combretaceae), roots and leaves of *Phyllanthus amarus* Wab (Beng. Bhui Amla) (Fam. Euphorbiaceae), whole plant of Euphorbia hirta L. (Beng. Dudhi) (Fam. Euphorbiaceae) flowers and fruits of *Bombax ceiba* L. (Beng. Shimul) (Fam. Bombacaceae), roots of *Rauwolfia Serpentina Benth.* (Beng. Sarpagandha) (Fam. Apocyanaceae), roots leaves and flowers of *Hibiscus rosa-simensis* L. (Beng. Jaba) (Fam. Malvaceae), whole plant of *Portulaca oleracea* L. (Beng. Nune shak) (Fam. Poportulacaceae) roots, leaves and flowers of *Adhatoda Vasica Nees* (Beng. Basak) (Fam. Acanthaceae), roots leaves, flowers barks and white latex of *Calotropis Procera* Br. (Beng. Akanda) (Fam. Asclepiadaceae), roots, flowers and fruits of *Baulhinia acuminita* L. (Beng. Kanchan) (Fam. Leguminosae), leaves of *Moghania macrophylla willd.* O. Kentz (Beng. Moghania) (Fam. Leguminosae), flowers and seeds of *Spilanthes acmella* L. (Beng. Marhatitiga) (Fam. compositae), roots, barks and seeds of *Bixo orellana* L. (Beng. Latkan) (Fam. Peperomiaceae), Leaves and flowers of *Leucas aspera*

willd (Beng. Dandakalas), leaves of *Tridax procumbens* L. (Beng. Tridhara) (Fam. Compositae); barks, fruits and flowers of *Phyllanthus emblica* L. (Beng. Amloki) (Euphorbiaceae), leaves of *Desmodium gangeticum* (L.) DC. (Beng. Salpani) (Fam. Leguminoase), whole plant of *Enhydra fluctuans* Lour. (Beng. Helencha) (Fam. Compositae) roots and leaves of *Scoparia dulcis L.* (Beng. Bandhania) (Fam. Scrophulariaceae), roots and barks of *Urena Sinuate L.* (Beng. Ban-okra) (Fam. Malvaceae) leaves and seeds of *Sesamum indicum* L. (Beng. Til) (Fam. Pedaliaceae), roots and leaves of *Polygomum orientale* L. (Beng. Biskantali) (Fam. Polygonaceae) [5]are used as medicine for the treatment of dysentery in Bangladesh.

Diuretic:

The barks of *Terminalia arjuna* [9] (Beng. Arjun, Arjuna), the fruits of *Eugenia jambolana* [9] (Beng. Cholojam, Jam) *Luffa aegytiaca* [9] Beng. Dhundul); the roots of *Bergenia ligulata* [9] (Beng. Phathorchuri, Pathorchuchi), *Lawsonia inermis* [9] (Beng. Mehidi, Mendi), the seeds of *Helianthus annus* [9] (Beng. Surjamuki), the leaves and plants of *Heliotropium indicum* [9] Linn (Beng. Hatirshoor, Hatishoor), the roots and fruits of *Abutilon indicum* [9] (Beng. poltari, Jhumko), the roots and leaves of *Asparagus racemosus* [9] (Beng. Shotomuli) etc. are used as diuretic agents.

Eczema:

Roots, leaves and stems of *Amaranthus spinosus* L. (Beng. Kantu notey) (Fam. Amaranthaceae), roots, leaves and seeds of *Achyranthes aspera L.* (Beng. Apang) (Fam. Amaranthacea); whole plant of *Peperomia pellucidu* Kunth (Beng. Luchipata) (Fam. Pepero miaceae), roots and leaves of *Cassia alata* L. (Beng. Dadmardan) (Fam. Leguminosae) [5] are used for the treatment of eczema.

General tonic:

The flowers of *Helianthus annus* [9] (Beng. Surjamukhi), *Rosa centifolia* [9] (beng. Golap), *Jasminium ambac* (Beng. Beli, Banmallika), the leaves and flowers of *Acasia arabica* (Beng. Babla), *Psidium guyava* [9] (Beng. Peyara, Piyara), the seeds of *Carica papaya*[9] (Beng. Papaya), *trigonella foenum graecum* [13] (Beng. Methi); the roots of *Bergenia ligulata* [9] (Beng. Patharkuchi), *Plumbago zeylanica* [9] (Beng. Chitruk), the fruits of *Terminalia chebula* [9] (Beng. Horitoki); the barks of *Terminalia arjuna* [9] (Beng. Arjun, Arjuna), (Fam. Combretaceae), the plants of *Vernonica cinerea* [9] (Bneg. Kalajira) etc. are used as general tonic.

Gonorrhoea:

The roots, leaves and flowers of *Coccinia cordifolia* L. Cogn. (Beng. Telakucha) (Fam. Cucurbitaceae), roots, leaves, barks and seeds of *Cassia occidentalis* L. (Beng. Bara Kalkaesunde) (Fam. Leguminosae), seeds and flowers of *Linium usitatissimum* L. (Beng. Tisi) (Fam. Linaceae), leaves of *Ipomoea aquantica Forsk.* (Beng. Kalmi shak) (Fam. Convolvulaceae), roots, leaves and seeds of *Clitoria ternatea* L. (Beng. Aporajita) (Fam. Leguminosae), leaves and barks of *Abroma augusta* L. (Beng. Ulatkambal) (Fam. Sterculiaceae), rhizomes of *Gloriosa superba* L. (Beng. Ulatchandal) (Fam. Liliaceae); roots and barks of *Urena sinuate* L. (Beng. Banokra) (Fam. Malvaceae), roots and leaves of *Phyllanthus amarus wab* (Beng. Bhui amla) (Fam. Euphobiaceae); tender leaves of *Comellia simensis* (L.) O. Kuntze (Beng. Cha) (Fam. Theaceae) leaves of *Premana integrifolia* Linn. (Beng. Goniari) (Fam.Verbenaceae); roots and fruits of *Solanum Zesembifolium* L. (Beng. Kantakini) (Fam. Solanaceae); roots, seeds, barks and leaves of *ClomeVicosa* L. (Beng. Hurharia) (Fam. Capparidaceae); leaves and fruits of *Physalis minima* L. (Beng. Phutki) (Fam. Solanaceae) and roots, leaves and stems of *Amaranthus spinosus* L. (Beng. Kanta notey) (Fam. Amaranthaceae)[9], the the leves of *Acacia arabica* [9] (Beng. Babla), the roots of *Lawsonia inermis* [9] (Beng. Mehidi, Mendi), *Ipomoea digitata* [7] (Beng. Bhui Kumra), the seeds and unripe fruits of *Abelmoschus esculentus* [9] (Beng. Dherosh) etc. are reported to be helpful for the treatment of gonorrhoea.

Hoemorrhage:

The barks of *Dalbergia Sisso Rixb* [6] (Beng. Sisu), *Desmodium Pulchellum* [6] (Beng. Jutasalpani), the leaves of *Asclepias curassavica* [9] Linn (Beng. Kakturi), the fruit of *Averrhoa bilimbi* Linn (Beng. Bilimbi), *Benincase hispida* [9] (Beng. Chalkumra) leaves and rhizomes of *Typhonium trilobatum* (L.) Scott (Beng. Ghetukachu) (Fam. Araceae), roots and leaves of *Pogostemon pubescence* Benth (Beng. Shul) (Fam. Labiatae), *Withania Somnifera* Dunal. (Beng. Aswangsndha) (Fam. Solanaceae) and the upper part of the plant of *Eupatorium triplinerve* Vahl. (Beng. Ayapan) (Fam. Compositae) [5] are used as medicines for chek haemorrhages.

Heart disease:

Leaves and fruits of *Solanum migrum L.* (Beng. Kakmachi) (Fam. Solanaceae), leaves and seeds of *Cajanus cajan (L.)* Mill (Beng. Arhar) (Fam. Leguminosae), fruits and barks of *Terminalia arjuna* Bedd (Beng. Arjun) (Fam. Combretaceae), upper part of the plant of *Eupatorium triplinerve Vahl.* (Beng. Ayapan) (Fam. *Compositae), roots and fruits of Carissa congesta* Wigth. (Beng. Karamcha) (Fam. Apocynaceae) and leaves of *Pandanus Odoratissimus* L.f. (Beng. Keya) (Fam. Pandanaceae) are used against heart diseases.

Hypertension:

The roots of *Rauwolfia Serpentina* [5](Beng. Sarpagandha) are known to be an important source of hypertensive and tranquillizer reserpine.

Jaundice:

Roots of *Asparagus racemousus L.* (Beng. Shatamuli) (Fam. Liliacease); fruits and barks of *Terminalia chebula tetz* (Beng. Haritoki) (Fam. Combretaceae) the leaves of *Aloe indica willd* (Beng. Grithakumari) (Fam. Liliaceae); roots leaves, fruits barks and seeds

of *Azadiracta indica L.* (Beng. Nim) (Fam. Meliaceae) roots and whole plant of *Boerhaavia diffusa L.* (Beng. Purnanaba) (Fam. Nyctaginaceae); barks, seeds and seed oil of *Carthamus tinctorius L.* (Beng. Kusum phul) (Fam. Compositae); roots and leaves of *Phylanthus amarus* Wab. (Beng. Bhui amla) (Fam. Euphorbiaceae); barks, fruits and flowers of *Phyllanthus emblica L.* (Beng. Amloki) (Fam. Euphorbiaceae); whole plant of *Hedyotis eorymbosa L.* (Beng. Khet papra) (Fam. Rubiaceae); whole plant of *Centilla asiatica (L.)* (Beng. Thankuni) (Fam. Umbelliferae); leaves, barks, fruits and seeds of *Flaceourtia indica Merr* (Beng. Baichi) (Fam. Flaceourtiaceae); leaves and seeds of *Cajanus cajan (L.) Mill* (Beng. Arhar) (Fam. Leguminosae); roots, barks and seeds of *Bixa orellana L.* (Beng. Latkan) (Fam. Peperomiaceae); leaves and flowers of *Leucas aspera (willd) Link.* (Beng. Ḍandakalas)[5]; the roots, leaves and flowers of *Coccinia indica*[9] (Beng. Telakucha) (Fam. Cucurbitaceae); the leaves and barks of *Lawsonia inermis*[9] (Beng. Mehendi); the plants of *Sphaeranthus indicus* [9] (Beng. Chagalnadi); the roots of *Ipomoea terpethum* [3] (Beng. Dud Kalmi) etc. are used as cure of jaundice.

Leprosy:

Barks and fruits of *Terminalia belerica* Roxb (Beng. Bohera) (Fam. *Combretaceae); roots leaves, flowers, barks and white latex of Calotropis procera Br.* (Beng. Akanda) (Fam. Asclepiadaceae); leaves, flower, fruits and seeds of *Lawsonia inermis L.* (Beng. Mehedi) (FAm. Lythraceae); flowers and fruits of *Bombax ceiba L.* (Beng. Shimul) (Fam. Bombacaceae); leaves, flowers and fruits of *Vernonia Patula (Dryand) Merr.* (Beng. Kooksim) (Fam. Compositae); leaves and seeds of *Cajanus cajan L. Mill* (Beng. Arhar) (Fam. Leguminnosae), whole plant of *Cymbopogon citrates (DC) stap. f.* (Beng. Lemon grass) (Fam. Gramineae); leaves of *Pandanus odoratissimus L. f.* (beng. Keya) (Fam. Pandanaceae); roots and barks of *Urena sinuate L.* (Beng. Ban okra) (Fam. Malvaceae) roots, leaves, flowers, barks and white latex of *Calotropis Procera Br.* (Beng. Akanda) (Fam. Asclepiadaceae) [5] are used as treatment of leprosy.

Malaria:

An infusion of the flowers of *Caesalpinia pulcherima* [6] (Beng. Krishnachura) in malerial fever, ground leaves of *Calycopteris floribunda* [6] *Linn* (Goachelata) leaves of *Helianthus annus* [6] (Beng. Surjamukhi), the decoction of *Lantana camera* [6] *Linn* (Beng. Chotra) etc. are considered useful in the treatment of malaria. Leaves flower and fruits of *Vernonia Patula (Dryand) Merr.* (Beng. Kooksim) (Fam. Compositae) [5], the whole plant of *Xanthium indicum Koen. ex Roxb* (Beng. Ghagra) (Fam. compositae), leaves and braks of *Vitex negundu L.* (Beng. Nisinda) (Fam. Verbenaceae), leaves, flowers, seeds and whole plant of *Ocimum Sanctum L.* (Beng. Tulshi) (Fam. labiatae), roots, leaves and flowers of *Adhatoda Vasica Nees* (Beng. Basak) (Fam. Acanthaceae) [5] are used for malaria in the rural areas of Bangladesh.

Piles:

The leaves of *Aloe indica* Willd (Beng. Grithakumari) (Fam. Liliaceae) fruits and barks of *Terminalia chebula* Tetz (Beng. Haritoki) (Fam. Combretaceae), fruits and barks of *Terminalia belerica Roxb* (Beng. Bohera) (Fam. Combretaceae), roots, fruits, barks and latex of *Ficus hispida L.* (Beng. Jagadumur) (Fam. Moraceae); leaves of *Paederia foetida L.* (Beng. Gandhabhadal) (Fam. Rubiaceae); leaves and seeds of *Cajanus cajan (L.) Mill* (Beng. Arhar) (Fam. Leguminosae); leaves and fruits of *Solanum nigrum L.* (Beng. Kakmachi) (Fam. Solanaceae); roots, flowers, barks and fruits of *Baulhinia acuminita L.* (Beng. Kanchan) (Fam. Leguminosae); leaves of *Jasminum Sambac (L.) Ait.* (Beng. Beli) (Fam. Oleaceae); leaves of *Desmondium gangeticum (L.) DC* (Beng. Salmpani) (Fam. Leguminosae); roots and leaves of *Withania Somnifera Dunal.* (Beng. Aswangandha) (Fam. Solanaceae); fruits of *Carum copticum Benth.* (Beng. Jowan) (Fam. Umbelliferae); roots leaves and seeds of *Achyranthes aspera L.* (Beng. Apang) (Fam. Amaranthaceae), leaves and seeds of *Sesamum indicum L.* (Beng. Til) (Fam. Pedaliaceae), whole plant of *MimosaPundica L.* (Beng. Lajjabti) (Fam. Leguminosae); leaves and fruits of *Solanum migrum* [5] *L.* (Beng. Kakmachi) (Fam. Solanaceae) are used against piles.

Rheumatism:

Barks and white latex of *Calotropis Procera Br.* (Beng. Akanda) (Fam. Asclepiadaceae); fruits and seeds of *Elettaria Cardamomum Maton* (Beng. Elache) (Fam. Zingiberaceae); fruits and barks of *Terminalia belerica* Roxb (Beng. Bohera) (Fam. Cobretaceae), roots and seeds of *Ricinus communis L.* (Beng. Bherenda) (Fam. Euphorbiaceae), roots, leaves and seeds of *Clitoria ternatea L.* (Beng. Aparajita) (Fam. Leguminosae); whole plant of *Centella asiatice L.* (Beng. Thankuni) (Fam. Umbellifera); barks and seeds of *Azadirachta indica Linn* (Beng. Neem) (Fam. Meliaceae); seeds and whole plant of *Hypitis suaveolens Poir* (Beng. Tokama) (Fam. Labiatae); leaves and flowers of *Leucas aspera (Willd)* (Beng. Dandakalas) (Fam. Labiatae); Rhizomes of *Acorus Calamus L.* (Beng. Boch) (Fam. Araceae); roots of *Hemidesmus indicus L.* (Beng. Anantamul) (Fam. Asclepiadaceae) whole plant of *Cymbopogon citrates* (Beng. Lemon grass) (Fam. Gramineae); seeds of *Trogonella foenum graceum L.*[5] (Beng. Methi) (Fam. Leguminosae); roots and leaves of *Withania somnifera Dunal* (Beng. Aswagandha) (Fam. Solanaceae) and seeds of *Nigella sativa* [5] *L.* (Beng. Kalojira). (Fam. Ranunculaceae), leaves of *Acanthus ilicifolius* [6] *Linn* (Beng. Harzora, Kotki, Harkuch), *Allium cepa*[6] *Linn* (Beng. Piyaj), *Cassia fistula* (Beng. Sonalu, Banderlathi, Sondal), *Citrulus Colocynthis* (Beng. Makal); *Dipterocarpus alatus* [6](Beng. Garjan, Shilgarjan, Dhuligarjan, Mashkalya-garjun) etc. are used against rheumatism.

Skin diseases:

The leaves and barks of *Lawsonia inermis* (Beng. Mehindi) *Hydrocotyl asiatica* (Beng. Thankuni) the barks of *Albizzia amara* (Beng. Amlaki), the plant of *Cynodon dactylon* [5] (Beng. Durba), the barks leaves and juice of ripe fruits of *Cassia fistula* [9] *Linn* (Beng. Bandar lathi) etc. are used as medicines for the treatment of skin diseases.

Syphilis:

Leaves and seeds of *Sesamum indicum L.* (Beng. Til) (Fam. Pedaliaceae), Leaves of *Pandanus odoratissimus L. f.* (Beng. Keya) (Fam. Pandanaceae); roots, leaves, flowers, barks and white latex of *Calotropis Procera Br.* (Beng. Akanda) (Fam. Asclepiadaceae); roots of *Hemidesmus indicus L. R. Br.* (Beng. Anantamul) (Fam. Asclepiadaceae) roots, leaves, barks and seeds of *Cassia occidentalis L.*(Beng. Bara Kalkaesunde) (Fam. Leguminosae)[5] are used as folklore medicines for the treatment of Syphilis.

Typhoid:

Barks and roots of *Croton oblonjifolius* (Beng. Chuka, Patri, Baragachi), *Celerodendrum inerme* (Beng. Bhat, Koklata, Banjui, Batrag, Bakri), *Desmodium gangeticum* (Beng. Salpani, Chaloni), *Grewia mierocos* (Beng. Asar, Patka), *Hedyotis corimbosa* (Beng. Khetpara), *Urarria logopoides*[6] etc. used for the treatment of typhoid (remittent).

Ulcers:

The leaves of *Psidium guyava* (Beng. Payara), *Lawsonia inermis* (Beng. Mehidi) the barks of *Acacia farnesiana* (Beng. Guya babla), *Acacia catechu* (Beng. Khoyer), *Eugenia jambolana* (Beng. Jam), *Punica granatum* [9] (Beng. Dalimgach), *Terminalia arjuna*(Beng. Aurjun); the plants and roots of *Ipomoea terpethum*[7] (Beng. Dud Kalmi), the leaves and fruits of *Areca catechu* [9] (Beng. Shupari) etc. are used as medicines for the treatment of ulcers.

1.3 : Studies on the medicinal plants and herbs of our country :

Attempts for phytochemical investigations on a large number of Bangladeshi medicinal plants and herbs including some of those already mentioned in the above section 1.2 were done by different groups of Chemists, Biochemists and Pharmachists of our country. But for want of advanced chemical and instrumental technologies most of these works are

either inadequate or preliminary in nature. Therefore, the medicinal plants and herbs of our country demands a thorough phytochemical, pharmacological and clinical investigations in a systematic manner for extraction, fractionation and isolation leading to the discovery of **usable drugs.**

The present work involves the phytochemical investigations on the leaves of *Tylophora indica* syn. *Tylophora asthmatica* W. & A. belonging to the genera *Tylophora* of the family **Asclepiadaceae.** As found in the literature, the following sections represent a brief review primarily on the various aspects of the species *indica* syn. a*sthmatica* of the genus *Tylophora* under the family **Asclepiadaceae**[12].

1.4 : The plant family Asclepiadaceae :

The plant family **Asclepiadaceae** consists of 320 genera comprising of 1700 species most of which are tropical while only a few of them are temperate. The plants of this are found to grow in Bangladesh, India, Sri-Lanka, Siam, Malay islands, Seychelle islands and Mauritius and Bourbon[13] and Robert Bentlley[14]. The members of this family are mostly herbs or shrubs frequently twining, often with milky juice. **Leaves** opposite or whorled, rarely alternate with stiples 0. **Flowers** hermaphrodite, regular, solitaryor many together, in umbels, umbellate cymes, fascicles or racemes, lateral or terminal.**Calyx** usually divided to the base; segments imbricate, usually with minute processes or glands at the base inside. **Corolla** hypogenous, gamopetalous, 5-lobed. **Ovary** superior, of 2 one-celled carpels enclosed within the staminal column, with their styles united above into a disk which is 5-angled. Fruits are two follicles (1 sometimes suppressed). Seeds are compressed, usually flat, often margined, crowned with a tuft of long hairs at one end [15]

1.5: The genus Tylophora and its distribution :

The plants or herbs of the genus *Tylophora* are perennial branching climber with fleshy roots and grow well in light sandy ground. Most of them are found growing wild in

almost all the plains of India. *Tylophora indica* syn. *Tylophora asthmatica* is the most important species of this genus. *Tylophora indica* is found to grow in the forests and hilly regions even upto the altitudes of 3,000 feet above the sea level throughout the Southern and Eastern parts of India. *Tylophora indica* grows abundantly in the North and East Bengal, Assam, Kachar, Chittagong and also in the Deccan peninsula[12]. There are as many as fourty (40) different species of *Tylophora* that grow in Bangladesh, India, Sri-Lanka, Siam, Malay islands, Seychelle islands and Mauritius and Bourbon[13] and Robert Bentlley[14]. Table-1.1 represents some of the important species of *Tylophora* growing abundantly in Bangladesh, India, Sri-Lanka and Mauritius[12,13,14,15].

Table 1.1 : *Tylophora* species growing in Bangladesh and in other countries of the world.

Family	Scientific name		Name of the countries where grow widely
	Genera	Species	
Asclepiadaceae	*Tylophora*	*indica*	Bangladesh, India and Sri-Lanka
Asclepiadaceae	*Tylophora*	*atrofolliculata*	"do"
Asclepiadaceae	*Tylophora*	*cordifolia*	"do"
Asclepiadaceae	*Tylophora*	*crebriflora*	"do"
Asclepiadaceae	*Tylophora*	*dalzellii*	"do"
Asclepiadaceae	*Tylophora*	*flava*	"do"
Asclepiadaceae	*Tylophora*	*floribunda*	"do"
Asclepiadaceae	*Tylophora*	*hirsuta*	"do"
Asclepiadaceae	*Tylophora*	*kerrii*	"do"
Asclepiadaceae	*Tylophora*	*mollissima*	"do"
Asclepiadaceae	*Tylophora*	*ovata*	"do"
Asclepiadaceae	*Tylophora*	*sylvatica*	"do"
Asclepiadaceae	*Tylophora*	*tanakae*	"do"

1.6 : The species *indica* Syn. *asthmatica* of the genus *Tylophora* :

The plant species *indica* is a perennial branching climber with long fleshy roots. Generally, it grows wild in the plain land or jungles of India. It can also grow in the forests and hiully regions upto a height of 3000 feet above the sea level throughout the southern and eastern parts of India. It grows very well particularly in the plain lands of Bangladesh, North Bengal, West Bengal, Assam, Kachar, and also in the hilly regions of Chiuttagong and Deccan penisula. The whole plant is of a pale yellow brown color and has no marked odor but has sweetish and subsequent acrid taste[12].

1.6.1: Botany of *Tylophora indica* :

Tylophora indica is a twining perennial herb, roots many, long, fleshy ; stems slender, twining, tortuous, terete, densely pubescent, at least when young, reaching 10 to 12 feet in length. Leaves opposite, on pedicels about ½ inch in length, spreading, blade 2-4 inches long, broadly ovate, rounded or cordate at the base, but with short mucro at the apex, quite entire, smooth above, usually downy beneath, thick, the upper narrower. Flowers small, numerous, on slender, bristly pedicels, ½ to ¾ inch long, arranged in irregular, umbellate, long-stalked panicles coming off from between the petioles; bracts rather, long linear. Calyx divided nearly to the base into five triangular-linear, striate segments with a few long white bristles on the outside. Corolla twice as long as calyx, spreading, divided about half way down into five broadly oval segments, dull orange or reddish. Stamens 5, inserted on the base of the corolla, erect, connected at the base, otherwise distinct though in contact, each united on the other side with the "corona", which consists of 5 distinct, fleshy bodies, broad and flattered below, and prolonged upwards into a narrow, acute, erect tongue about as long as the stamens ; anthercells and pollinia small, horizontal. Pistil of two carels, ovaries and styles distinct, stigma single, capitate, with a rounded, prominent centre, and a five radiating lobes in contact with the antthercells. Fruit of two ovoid, acuminate smooth follicles, 3 to 4 inches long and widely spreading. Seeds numerous, comose[14].

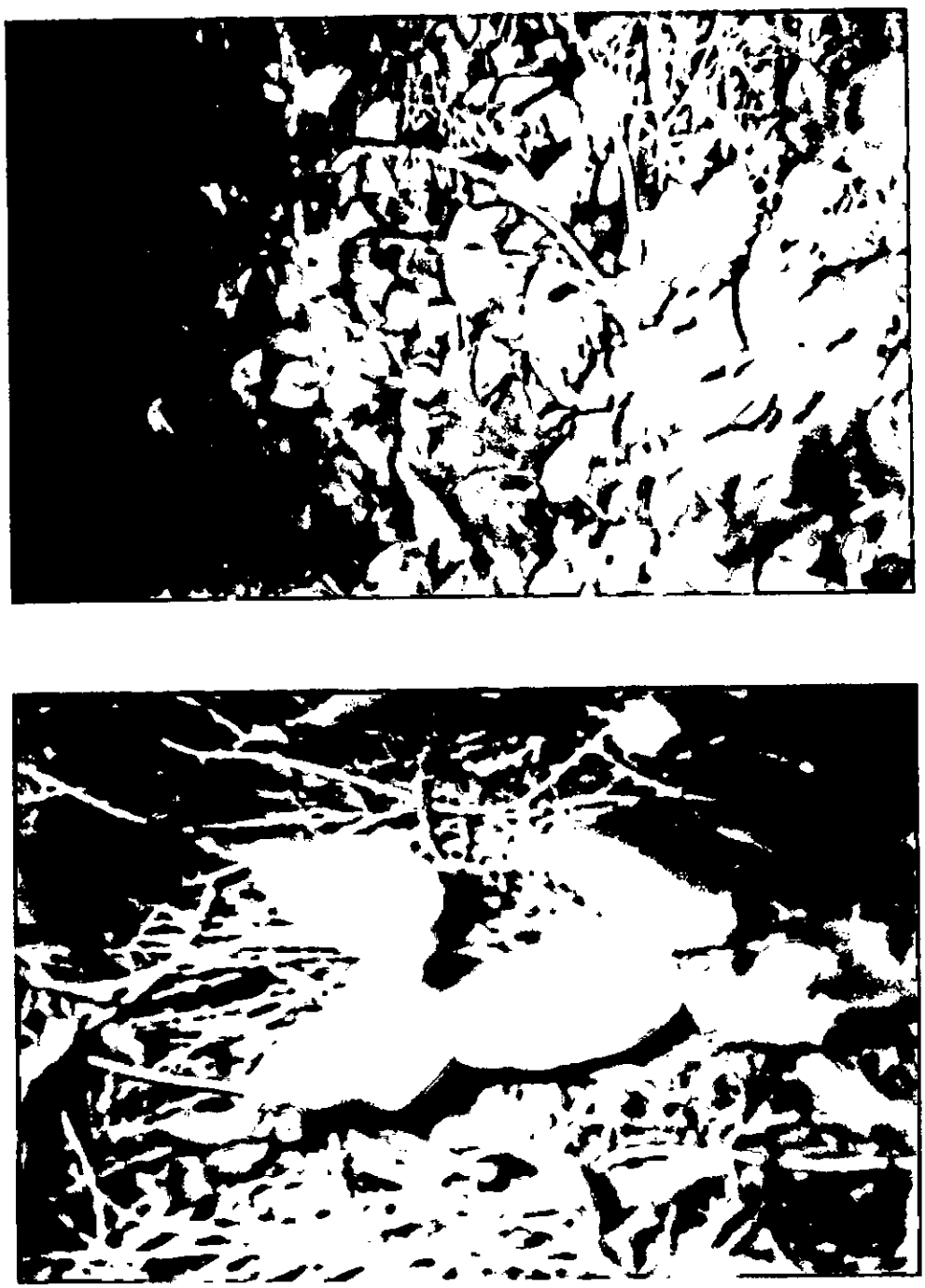


Fig.1.1: A section of *T. indica*

1.6.2 : Characters and composition of the leaves of *Tylophora indica* :

In the phermacopoeia of India, the characters of the dry leaves are given as follows :- From two to three inches in length, entire, ovate-roundish, acuminated, cordfate at the base, glabrous above, downy beneath. They have heavy disagreeable smell when bruised, and nauseous taste[14].

No complete analysis of the leaves has been done, but the authors of pharmacograhia stated that concentrated infusion is " abundantly precipitated by tannic acid, by neutral acetate of lead or caustic potash, and is turned greenish-black by perchloride of iron".Broughton obtained some crystals from the leaves and the " crystals when dissolved and injected into a small dog, they occasioned purging and vomiting[14].

1.6.3 : Medicinal properties and uses of *Tylophora indica* :

The medicinal properties of the plant have long been known to the natives of the parts where it grows, and have had seriously attracted the attention of the indigenous physicians. It is, however, not mentioned in any of the standard Sanskrit or Mohammedan works on Materia Medica but was a household remedy first brought into the notice of the western medicine by Roxburgh. Both the roots and leaves of the plant have often been employed as a substitute for ipecacuanha and very favourable reports as regards to its efficacy were given by Roxburgh, Ainslie, O`Shaughnessy, Dobson and others. In large doses it acts as emetic and in smaller doses, often repated as cathartic. According to O`Shaughnessy the emetic properties of the root wre well established, but it was necessary to prescribe in doses double those of ipecacuanha, for which it was considered to be an excellent substitute. As regards the medicinal properties of the *Tylophora indica* Dr. J. Kirkpatrick commented, " I have administered this medicine in at least a thousand cases, and found it most valuable. In dysentery and as a simple emetic, it is in every way comparable to ipecacuanha. The dose is from 20 to 30 grains with half a grain or a grain of tartar emetic, if a strong emesis is required. If the dysentery distinctly

arise from intermittent disease, the quinine is conjoined. The form of the medicine I use is the powder of the dry leaf. In catarrhal and chronic coughs it seems to act well." The value of this remedy was testified by so many practitioners in India and by dint of its well-marked emetic properties it was admitted as an official drug in the Bengal permacopoeia of 1844. The dried leaves were made official as they were found to be more uniform and certain in their actions than the roots. The leaves were described as one of the best indigenous substitutes for ipecacuanha and were recommended as useful in all cases indicating necessity of emesis and as a remedy for dysentery, asthma, catarrh and other affections, in which ipecacuanha is generally employed. The dose as as an emetic is from 25 or 30 grains of the powdered dried leaves and as a diaphoretic and expectorant from 3 to 5 grains thrice daily. The plant is also used extensively in Mauritius, where it is known as *Ipeca du pays* or *Ipeca sauvage*[12,14].

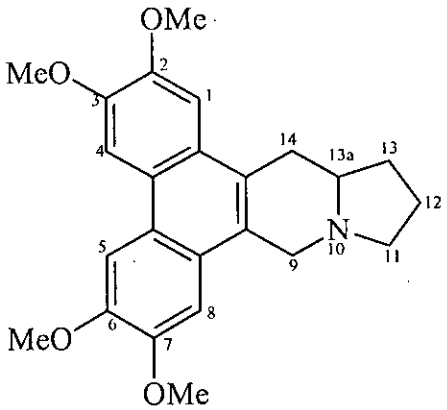
1.6.4 : Pharmacological activity of *Tylophora indica* :

In 1935 Ratangiriswaran and Venkatachalam while working continuously on the extraction and isolation of the alkaloidal fractions from the plant noticed that one of them got dermatitis. The effect was particularly observable when working with the solutions of the alkaloids in volatile organic solvents such as ether, chloroform and benzene. Aqueous acid solutions were not found to be so much active. The eruption appeared on the skin a day after exposure the first symptoms being itching with subsequent redness. Skin of the face became red and the eyelids and surrounding tissues were markedly swollen. There was exudation of serous fluid from the cracks that had formed on the skin. The symptoms continued for about a week and then gradually subsided. Simultaneously, disquamation occurred in the form of small scales and large flakes of of dried epidermis. The condition was relieved by moist compresses and the application of usual soothing lotions. In 1934 Richards and Lynn reported the occurrence of dermatitis with symptoms similar to those described above due to contact with leaves of *Ceanothus velutinus*, also an alkaloid containing plant though of a different family. The alkaloid Tylophorine is toxic to *pharmecium caudatum* in concentration of 1 in 50,000 or more. The toxicity of the alkaloid which varies with different species of

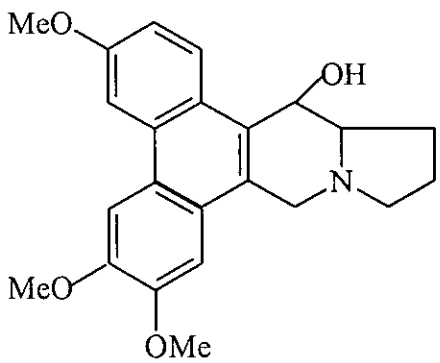
animals was worked out. The m.i.d. for frogs is 0.4 mg. Per gm. of body weight but its toxicity for mice and guinea pigs is very low. The alkaloid has no irritant action locally on the conjunction or on the skin. When injected subcutaneously or intramuscularly it produces little or no local reactions[12]. From the experimental data obtained it would appear that the effect of the drug is especially marked on the musculature of the body. The action on the cardiac muscle is however different, the having distinct depressive effect on the heart. The blood pressure is lowered when a dose is administered, but is raised soon after and is maintained at a level higher than the normal for a fairly long time. The initial fall is due to the depressant effect of the drug on the cardiac muscle and the subsequent rise to the stimulant effect on the plain muscles of the blood vessels resulting in contraction and increased cardiac output. In cardimeter experiments there is distinct evidence of decrease of both the systolic and diastolic phases of the heart. In mycaridograph experiments the amplitude of both the auricular and ventricular contraction was decreased. This is probably due to the direct effect of the drug on the cardiac muscalature and cannot be abolished by paralyzing the vagal endings with atropine. The absence of any effect of the drug on the pupil is explained by the fact that the two sets of muscle fibres in the iris , the circular and the radial, are antagonistic to each other and the stimulant effect on the one counter-balances that on the other. As a result of this the pupil remains unaffected [12].

1.6.5 : A brief review on the phytochemical investigations of *T. indica* :

Hooper (1891) reported the presence of a crystalline alkaloid, Tylophorine (1), in the roots of the plant *T. indica* and described some of its characteristic colour reactions, but the quantity isolated by him was not enough for complete analysis [16]. Ratnagiriswaran and Venkatachalam (1935) investigated the plant and isolated two crystalline alkaloids named Tylophorine, $C_{24}H_{27}NO_4$, m.p. 284-85°C (1) and Tylophorinine $C_{23}H_{27}NO_4$, m.p. 232-33°C [17]. In 1954, Govindachari et al. also isolated the alkaloids Tylophorine and Tylophorinine from *T.indica* and assigned the stuructures (1) and (2) for them[18] and later reported their syntheses[19-22].



Tylophorine (1)



Tylophorinine (2)

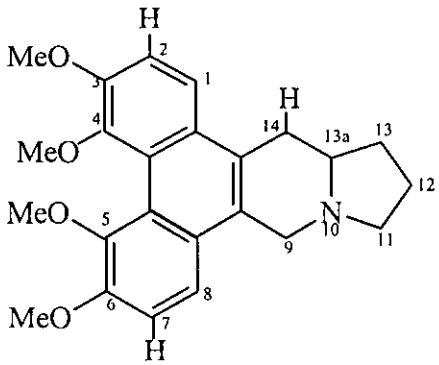
In 1971 Koppaka et al.[23] isolated five alkaloids from *T.indica* two of which were identified as tylophorine (1) and tylophorinine (2). The remaining three were new and were designated as A, B and C. Alkaloid A has two phenolic groups and two methoxyls. Alkaloid B has three methoxyls and one phenolic hydroxyl which on methylation with diazomethane yield yielded Tylophorine (1). The alkaloid C has two methoxyls, one phenolic hydroxy and one benzylic hydroxyl. Though these three alkaloids A, B and C had the same skeletal structures of tylopohorine and Tylophorinine (1) and (2) previously isolated, Koppaka et al.[23] could not assign the positions of the functional groups found in them. Of these five alkaloids *T.indica*, the new alkaloid C (tentatively called Desmethyltylophorinine) showed significant activity in murine leukemia (L-1210 system, Table 1.2).

Table 1.2 : Antileukemic activity new alkaloid Desmethyltylophorinine (C)

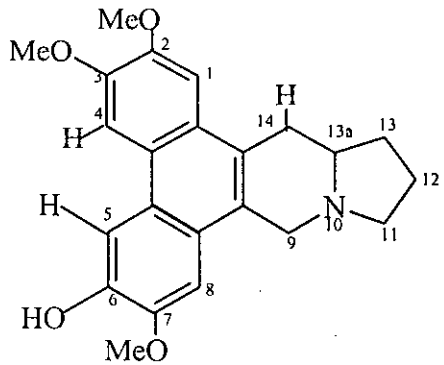
Dose, mg/kg	Change in Body weight	Increase in Survial Time %
12	-2.2	135
8	-1.5	149
6	+0.2	145
4	+1.3	138
2.7	+2.3	123

* An increase in survival rate of 125% or higher is considered as positive activity

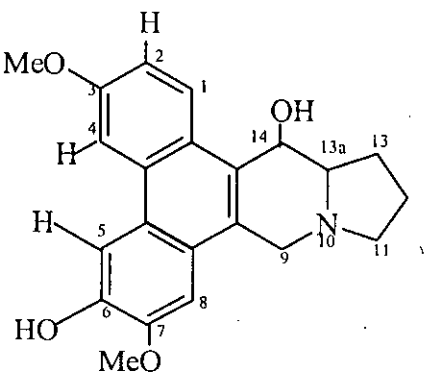
M.Ali and K.K.Bhutan[24] isolated 11 alkaloids from *T.indica* of whcich Tylophorine (1), 6-desmethyltylophorine (4), Tylophorinidine(5), 5-Hydroxy-O-methyltylophorinidine(6) were previously isolated from *T.Indica* by various workers and the rest seven Tyloindicine A (3), Tyloindicine-B (7), 14-Hydroxyisotylocrebrine (8), 4,6-Desdimethylisotylocrebrine (9), Tyloindicine C (10), Tyloindicine D(11), Tyloindicine E (12) were new.



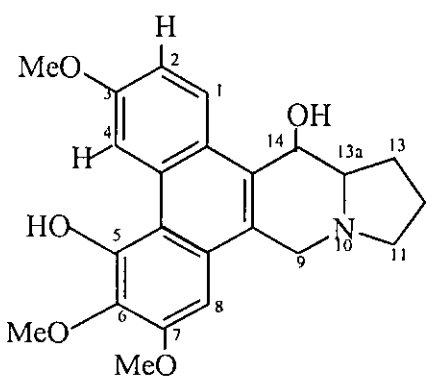
Tyloindicine A (3)



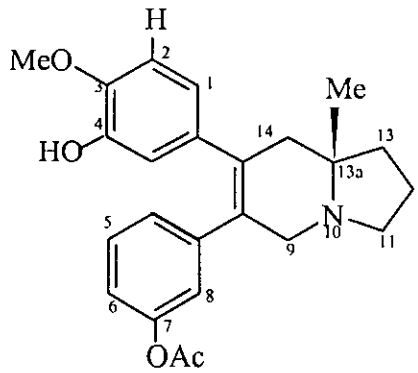
6-desmethyltylophorine (4)



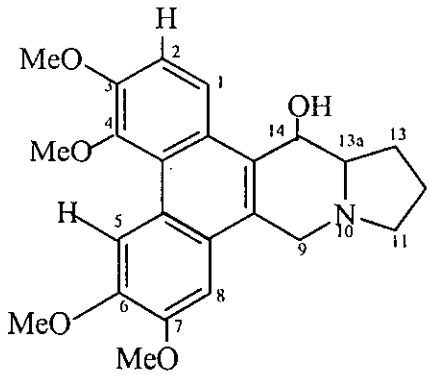
Tylophorinidine (5)



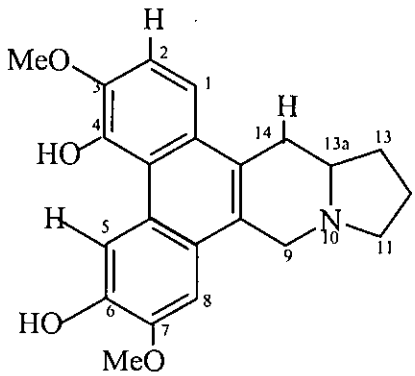
5-hydroxy-O-methyltylophorinidine(



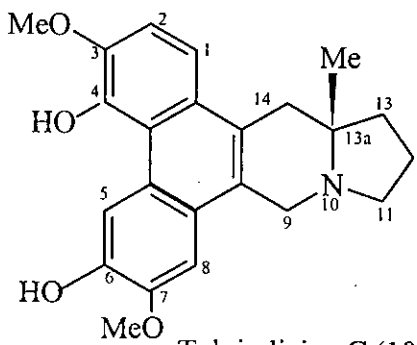
Tyloindicine B (7)



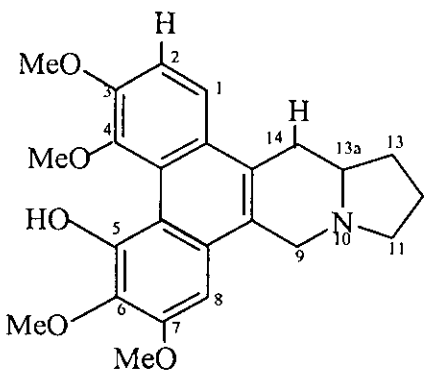
14-Hydroxyisotylocrebrine (8)



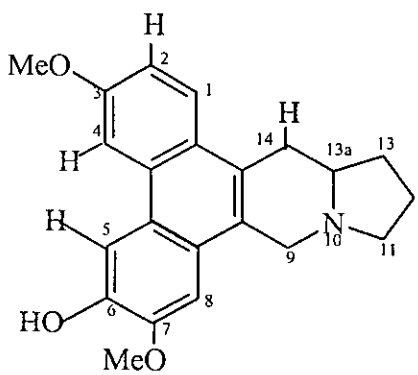
4,6-Desdimethylisotylocrebrine (9)



Tyloindicine C (10)



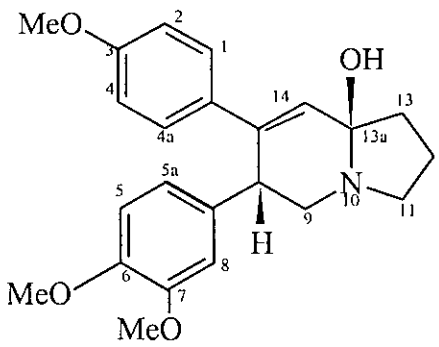
Tyloindicine D (11)



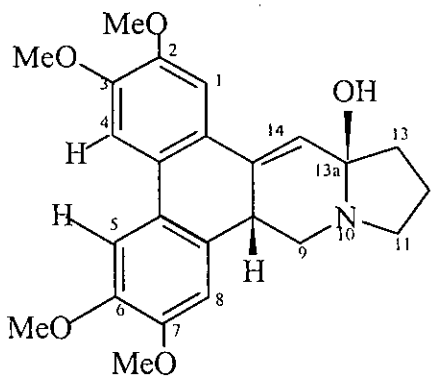
Tyloindicine E (12)

M. Ali et al. isolated another 5 new Tyloindicine phenanthroindolizidine alkaloids Tyloindicine F (13), Tyloindicine G (14), Tyloindicine H (15), Tyloindicine I (16) and Tyloindicine J (17) from the aerial parts of T. indica[25]. In addition to these 5 new

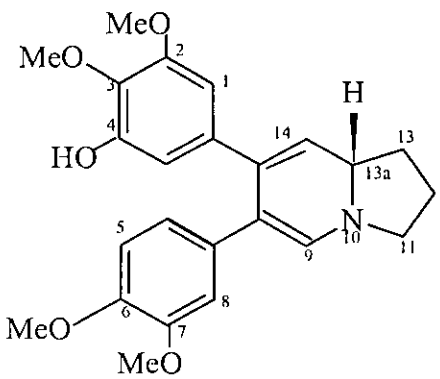
tyloindicines they also reported the isolation of a substituted phenanthrene hydrocarbon Tyloindane (18) along with Tylophorine (1). They assigned the structures of all these alksloids on the the basis of spectral analyses and chemical reactions.



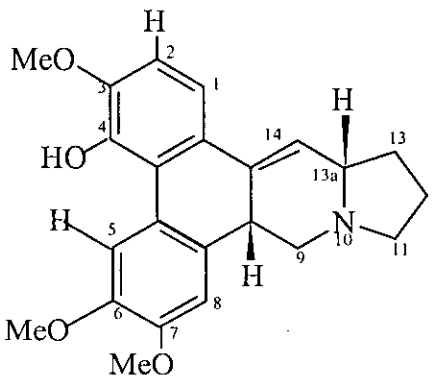
Tyloindicine F (13)



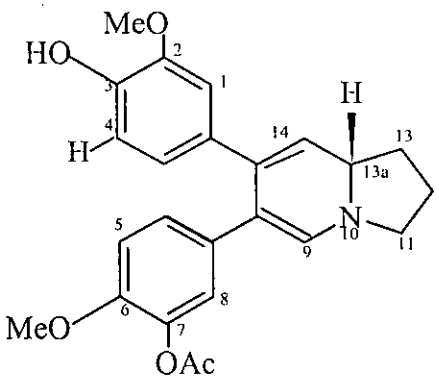
Tyloindicine G (14)



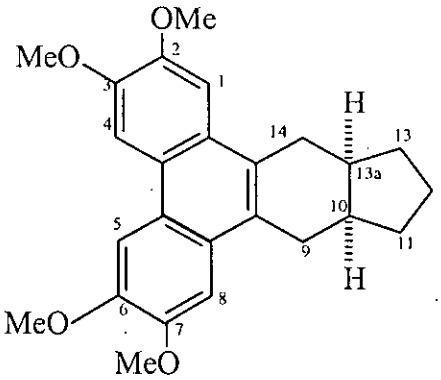
Tyloindicine I (16)



Tyloindicine H(15)



Tyloindicine J (17)



Tyloindane J (18)

Apart from alkaloids the plant *T.indica* also contains cetyl alcohol, phytosterol m.p 192-93°C , a newtrol substance of an alcholic nature m.p. 89-90 °C, a wax, a resin, chlorophyll, coloring matter, tannin, glucose, calcium salts and potassium chloride[12].

1.7 : The aim of the present work :

The present project has been undertaken for a detail investigation of the leaves of **anthamul** (***Tylophora indica***) with an aim for isolation, separation, purification and structural elucidation of the various compounds present there. Since quite a large number of alkaloids have already been isolated principally from its stems and roots and also from its aerial parts, it is quite reasonable that its leaves should also contain alkaloids amongst other compounds. Alkaloids being physiologically active compounds, our major objective is to isolate, separate and purify alkaloidal compounds from the leaves of anthamul and to determine the molecular architecture of the isolated alkaloids along with other compounds if any by chemical, physical and spectroscopic methods.

2.0: CHAPTER 2

2.1 : General methods :

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

2.1.1 : Preparation of extracts :

The plants powder was extracted exhaustively with organic solvents of increasing polarity e.g. with petroleum ether (40-60°C), Chloroform ($CHCl_3$), Ethyl acetate (EtOAC), Methanol (MeOH) and Rectified spirit (EtOH)

2.1.2 : Evaporation and concentration :

All evaporations and concentrations were done by rotary vacuum evaporation under reduced pressure at bath temperatures $\leq 40^{\circ}$ C. Small volumes of nonaqueous solvents such as chloroform ($CHCl_3$) and dichloromethane (CH_2Cl_2) were concentrated or evaporated by blowing dry nitrogen through the solvents at room temperature.

2.1.3 : Determination of melting points :

All melting points were recorded in a Fisher John`s Electrothermal melting point apparatus (Model no. 1A 9000) and was uncorrected. The heating was done carefully in order to maintain a uniform and a steady temperature.

2.1..4 : Centrifugation :

All centrifugations were carried out in a **Hettich universal 16A** centrifuge at 4000 rpm for a period of at least 20 minutes.

2.1.5 :Crystallization and Fractional crystallization :

The techniques of crystallization and recrystallization are used for purification of column chromatographic(CC) and vacuum liquid chromatographic (VLC) separated fractions. In the technique of crystallization generally a solvent is chosen in which the substance or the separated crude mass is least soluble. The compound or the crude mass obtained from chromatographic separation is clearly dissolved in a minimum volume of the solvent at an elevated temperature (if necessary filtered or centrifuged to make a clean solutuion) and left undisturbed at room tempertature or cooled in ice or kept in a refrigerator for crystallization or fractional crystallization. In some cases, especially in case of fractional crystallization usually a mixture of solvents are used. During fractional crystallization, the compound is usually dissolved in a suitable solvent and then a second solvent in which the compound is either insoluble or sparingly soluble is slowly added until cloudiness is appeared. Then it is left undisturbed at room temperature or cooled in ice or kept in a refrigerator for crystallization. When a batch of crystals are formed, it is isolated either by decantation or filtration. In order to remove the adhering materials, the isolated crystals are washed very quickly with the solvent in which it is soluble. The washings are are added to the mother liquors from decantation or filtration, concentrated and and left for a second batch of crystallization.

2.1.6 :Solvents and chemicals :

All the solvents and chemicals used in the extractions and experiments were procured from E.Merk (Germany) or BDH (England) or Aldrich (America) and were either meant for laboratory use or were of analytical reagent grade. All solvents, particularly solvents of commercial grades like dichloromethane, chloroform, ethyl acetate , methanol and rectified spirit were distilled prior to use for extraction, chromatographic separation or any analytical purpose. Before distillation pyridine was dried over phosphorus pentoxide and the distillate boiling at 115°C was collected over potassium hydroxide pellets. Before use pyridine was finally dried over molecular sieve.

2.1.7 : Chromatographic techniques :

In order to separate the crude extracts (from various solvent extraction) into the individual pure components, various types of chromatographic technique's were employed e.g. paper chromatography (PC), Vacuum liquid chromatography (VLC), Thin layer chromatography (TLC) preparative thin layer chromatography (PTLC) and Gas liquid chromatography (GLC).

2.1.7.1: Paper Chromatography : Paper chromatograms 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	(10:03: 03)
B.	n-Butanol	:	Ethanol	:	Water	(40:11:19)
C.	n-Butanol	:	Acetic acid	:	Water	(06:02: 01)
D.	Ethyl acetate	:	Acetic acid	:	Water	(03:01:01)
E.	Ethyl acetate	:	Pyridine	:	Water	(10:04:03)

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

(A) The irrigated dried papers are dipped in aqueous saturated solution of silver nitrate (1 ml) diluted with acetone (500 ml) immediately followed by dipping in ethanolic solution of sodium hydroxide (0.5M). These are then washed with 2% sodium thiosulphate solution followed by water.

(B) The irrigated dried papers are sprayed with an alcoholic solution of aniline oxalate (1%) followed by heating at 120° for 10 minutes.

(C) The irrigated dried papers are sprayed with an alcoholic solution of P-anisidine and Phathalic acid followed by heating at 100°C for 10 minutes.

(D) The irrigated dried papers were sprayed with ninhydrin for identificastion of amino acids .

2.1.7.2: Vacuum liquid chromatography (VLC) :

The concept of vacuum liquid chromato-graphy (VLC) is a recent development in the field of chromatographic separation*. It is a type of column chromatography done under reduced pressure and the column is usually packed with TLC grade silica gel. The advantage of this new technique is that it fractionates or splits the crude extracts either into pure compounds or mixtures of compounds containing less number of components.

In this technique a glass tube of about of 25-30 cm in length and 3-5 cm in diameter fitted with a water pump and a collecting flask at the bottom is used (Fig.2.1). Fine silica gel G-60, GF_{254} (E Mark, 7731) is used as an adsorbent.The gel is packed into the column under an applied vacuum in such a way so that a bed of about 5-7 cm height is obtained. The mixture to be separated is then pre-adsorbed in the required amount of the same gel (as packed in the column) and is placed on the top of the bed. Alternatively a clean concentrated solution of the extract or the mixture may employed on the top of the bed. A gradient elution is then carried out with solvents of increasing polarity until more polar components of the mixture or extracts are eluted. The effluents are collected manually in fractions of about 15-20 ml in test tubes and then the eluates from the tubes are monitored by TLC. The fractions showing 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 chromatographic separation lies in the fact that it requires less amounts of eluting solvents, minimum quantity of solid adsorbents and a shorter time.

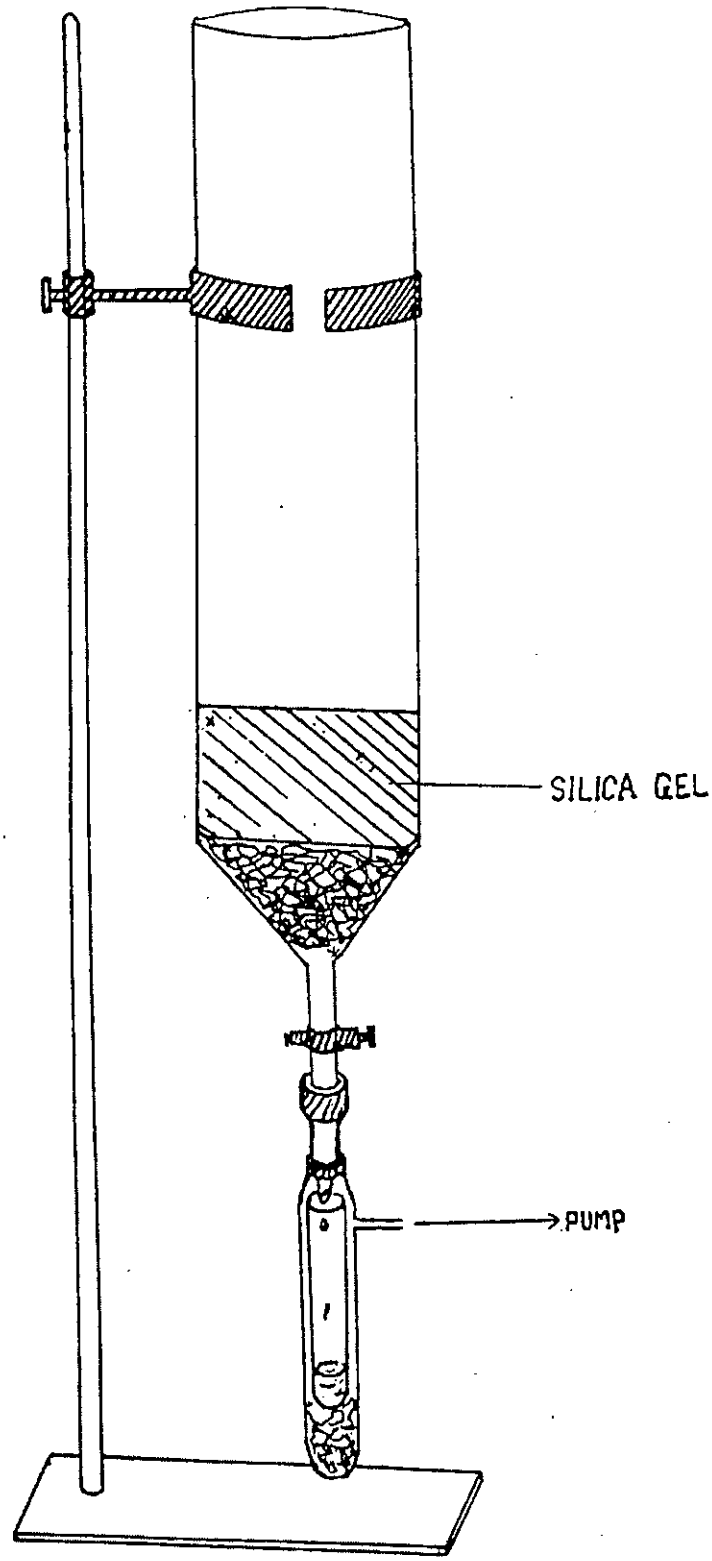


Fig. 2.1 : Vacuum liquid chromatography (VLC)

2.1.7.3 :Thin layer chromatography (TLC) :

During the entire course of the experiments two types of plates e.g. precoated TLC plates and manually prepared plates were used.

(i) Pre-coated TLC plates : A 0.2 mm thin coating of silica gel (60 GF_{254}) on aluminium sheets or glass plates were used.

(ii) Sometimes manually prepared silica gel plates were also used.

(a)**Manual preparation of the silica gel plates :** Glass plastes (20 x 20 cm ; 6 x 2 cm) were washed with detergent, wate, distilled water and finally with acetone. Special care was taken in handling the tubes to avoid any contamination. The glass plates were then spreaded with slurry of silica gel G-60, GF_{254} (E Mark, 7731) in distilled water (1:2) to have a layer of ~ 0.2 mm in thickness to act as a stationary phase. The plates so prepared were dried in the air. Finally the air dried plates were activated by heating them at 100°C in an oven for about an hour followed by cooling at room temperature.

(b)**Application of the samples and development of the plates :** Capillary tubes were used for spotting the samples on the plates. The spotted plates were developed by the ascending technique in TLC tanks using selected solvent systems.

(c)**Solvent systems :** The solvents of various polarities used in thin layer chromatography are given below :

(1) The following binary solvent systems were used for low polar compounds and fractions e.g.

(i) n-hexane : chloroform (in different ratios)
(ii) Chloroform : ethyl acetate (in different ratios)
(iii) Chloroform : methanol (in different ratios)

(iv) Ethyl acetate : methanol (in different ratios)

(2) Ternary solvent systems were used for more polar compounds and fractions e.g

(i) Chloroform : ethyl acetate : methanol (in different ratios)

(d) Detection of compounds on the developed chromatograms:

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

(i) UV light :

The compounds on the developed and dried TLC and PTLC plates were viewed under UV light source with two different wave lengths e.g. 254 nm and 350 nm. Some of the compounds were found fluorescing while the others were seen as darks spots of different colours.

(ii) Iodine vapour :

Iodine vapor is avery common and a versatile reagent for identifying compounds in developed chromatoplates. The plates were placed into the tank of iodine vapor to locate the spots.

(iii) Vanillin sulfuric acid spray :

The plates were sprayed with 1% solution of vanillin in concentrated sulfuric acid and the sprayed plates are heated at 110°C for 10 minutes to identify the spots. The compounds were identified on account of the development of specific colour (Mathews, 1963).

(iv) Potassium permenganate spray :

The chromatogram was sprayed with a 0.5% potassium permenganate reagent. The resolved compounds were identified with the development of colour instantly.

(v) Dragendorff's reagent :

The presence of an alkaloidal compound is usually detected by spraying the developed chromatoplates with Dragendorff's reagent when an orange red-spot is appeared.

e) The R_f value :

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

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

R_f value in a solvent system is a constant for any compound and it is a physical property of that compound [26].

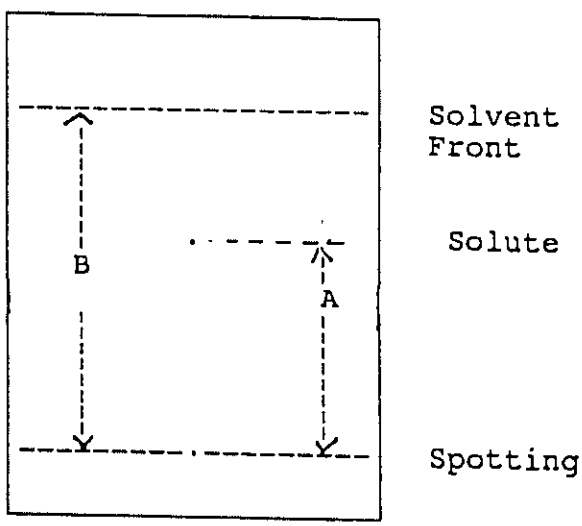


Fig. 2.2 : A TLC plate showing calculation of R_f value

(f) **Preparation of reagents including spray reagents for chromatograms :**

(i) **Vanillin sulphuric acid reagent :** Sulphuric acid (400 ml) and absolute alcohol (150 ml) is mixed in a beaker (kept in an ice bath). Vannilin (0.25 g) is added to this mixture of alcohol and sulphuric acid, cooled. Thus vannilin sulphuric acid reagent was prepared.

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

(iii) **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).

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

(v) **Mayer's reagent :** Mercuric chloride (1.358 g) was dissolved in distilled water (60 ml) and was poured into a solution of potassium iodide (5 g) in distilled water (10 ml).The volume of the solution was made 100 ml by adding required amount of water.

2.1.7.4: Column chromatography :

(a) **Column :** Glass tubes of different lengths and diameters e.g. 90 cm x 8 cm, i.d. and 60 cm x 3 cm i.d. and 30 cm x 1 cm i.d. fitted with a rota-flow control system were used column chromatographic separation.

(b) **Stationary phase :** For normal column chromatographic separation, silica gel G_{60}-GF_{254} was used as stationary phase.

(c) **Preparation of normal silica gel column :** To prepare a particular column, the required amount of silica gel is swelled into a selected solvent e.g. n-hexane, chloroform, dichloromethane, ethyl acetate or in a mixture of different solvents in different ratios and then poured into the column with continous flow of the solvent. For homogenous packing, the column is equilibrated with two or three column vcolume of solvent. Normal phase column chromatographic separation is usually performed by gravitational flow with solvents of increasing polarity.

(d) **Application of sample into the column :** The crude extract or a subfraction or a mixture of compounds is applied into the column either in a powdered from or as a solution. To prepare the powder form of the sample, the sample is dissolved in a particular solvent or in a mixture of solvents and silica gel (sample : gel = 1 : 2 w/w) is added to the sample solution and the mixture is finally evaporated to dryness falling into lumps. The dried lumps are thoroughly powdered in a mortar and the powder so obtained is applied on the top of the column.

For application of the sample in the form of solution, it is dissolved in a minimum volume of the column equilibrating solvent and very slowly added on the top of the equilibrated column with the help of a dropper. The sample layer is then leveled by gentle tapping of the column. On the top of this layer about o.5-1 cm of the silica gel was placed so that the surface of the bed is not affected during solvent application.

(e) **Fractionationation and monitoring procedure :** After sample application, the column is eluted with the equilibrating solvent and the polarity of the mobile phase is gradually increased by adding hexane, dichloromethane, ethyl acetate and methanol. The eluted effluents are collected either in conical flasks or test tubes. The fractions are monitored by TLC.The fractions having same R_f values are pooled together, concentrated and is kept for crystallization or fractional crystallization.

2.1.8 :Test for steroids :

(a) The Salkowski Test for steroid: The extracted substance (~2 mg) was taken in a test tube containing in a mixture of chloroform and methanol (~2 ml) and a few drops of concentrated sulphuric acid was slowly added from the side of the tube. Development of a reddish color in the chloroform layer indicates the presence of a steroid in the sample.

(b) The Liebermann-Burchard Test for steroids:

The extracted sample (~2mg) was taken in a test tube containing a mixture of chloroform and methanol and few drops of concentrated sulphuric acid.The development of a greenish color which turns blue on standing indicates the presence of a steroid in the sample

2.1.9 : Tests for sugars:

(a) Phenol-sulphuric acid Test for sugars:

Sample solution (0.5) was 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) was 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) was added directly and quickly into the sample using a dispenser.Sufficient care was taken to avoid splashing of any acid out of the tube.Development of a reddish brown color confirms the presence of a sugar in the sample.

(b) Molisch's Test for sugars:

1% sample solution (0.5 ml) was taken in a test tube and two drops of the Molisch's test reagent was added to it.The test tube was taken inclined and with a dropper concentrated sulphuric acid (1.0 ml) was carefully added so that it flowed down the side of the tube and formed a layer beneath the aquous solution. After a few minutes a red-violet ring or coloration was seen at the interface of the two layers. Gentle agitation, but not enough to mix the layers, caused the violet color to diffuse throughout the lower layer . For comparison, a parallel test with a pentose, hexose or a disaccharide was done.

Molisch's Test reagent :

α-napthol (2.0 g) was dissolved in 95% ethanol or rectified spirit (50 ml) to give the reagent.

2.1.10 : Spectroscopic methods :

(a) Infrared (IR) spectroscopy : Infra red spectra were recorded on SHIMADZU FTIR-8400-9000 spectrometer as KBr disk in the chemistry dept of Bangladesh university of eng and technology(BUET) Dhaka, Bangladesh.

(b) Nuclear magnetic resonance (NMR) spectroscopy: ^{1}H- nmr and ^{13}C-nmr spectra of some of the samples were recorded in $CDCl_3$ on a 400MHz spectrometer at the Dhaka laboratories of Bangladesh council of scientific and industrial research (BCSIR) Dhaka ,Bangladesh. ^{1}H- nmr and ^{13}C-nmr spectra of some other samples were recorded in a Bruker WH:-500MHz in the Tokyo Metropolitan University of Japan by Professor Md Abul Hashem.

(c) Mass spectroscopy: The mass spectra of the compounds EA-2, EA-3 and EA-4 were recorded in a high powered mass spectrometer in the Tokyo Metropolitan University of Japan by Professor Md. Abul Hashem.

3.0 : CHAPTER 3

3.1 : Collection of the leaves of *Tylophora indica* :

The climbing perennial herb *Tylophora indica* was cultivated in sufficient quantities in the Club premises of the Bangladesh University of Engineering and Technology (BUET), Dhaka. For the experimental purpose only the matured, fresh and sound leaves were collected during the month of October, 2001. The leaves were correctly identified as the leaves of *Tylophora indica* in the Bangladesh National Herbarium (BNH), Mirpur, Dhaka.

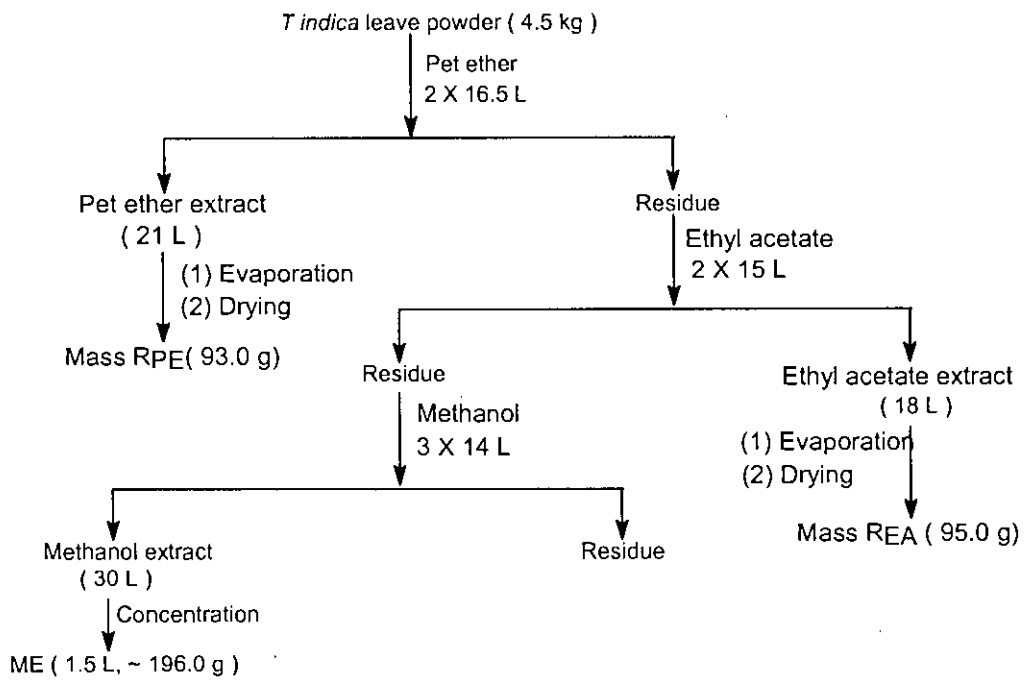
3.2 : Drying and grinding of the leaves :

The collected leaves were dried in the air (under a ceiling fan) in absence of sunlight and the dried leaves were grinded into powder in a cyclotech grinding machine (200 mesh) (4.5 kg). The powder was stored in polythene packets until used for extraction.

3.3 : Extraction and fractionation of the powder obtained from the leaves of *Tylophora indica* :

The dried powder was taken in 3 aspirator bottles each of capacity 6 litres (3 X 1.5 = 4.5 kg). Petroleum ether (40-60°C, ~ 5.5 L) was added in each of the three bottles so that the powder gets immersed under the petroleum ether, the level of petroleum ether being at least 3 cm. above the powder level. The extraction mixtures in the three bottles were allowed to stand at room temperature with frequent shaking by a dry wooden rod. After ~ 72 hours the extracts from the three bottles were collected and filtered through a fine cloth followed by filtration through Whatman no. 1 filter paper. The process of extraction with petroleum ether was repeated for the 2nd time in the same way and all the extracts were pooled together (~ 21 L). The left over residue from petroleum ether extract was successively extracted twice with ethyl acetate (EtOAc) and the ethyl acetate extract free residue was extracted 3 times with methanol at room temperature following exactly the same procedure as described for petroleum ether extraction and the filtered extracts obtained from Ethyl acetate (~ 18 L) and Methanol (~ 30 L) were collected separately.

The solvents from the Petroleum ether extract and the Ethyl acetate extract were evaporated in a rotary evaporator under reduced pressure at a bath temperature (≤ 40° C). These were then dried over phosphorus pentaoxide in a vacuum desiccator under vacuum. The petroleum ether extract on drying gave a greenish gummy mass named as R_{PE} (93 g). The ethyl acetate extract on drying also gave a greenish gummy mass named as R_{EA} (95 g). In the similar process, the methanol extract was concentrated (~1.5 L) when some very fine crystals were noticed in the walls of the evaporating flask. So, instead of further evaporation, the methanol extract named as ME (~ 1.5 L) was allowed to stand in a refrigerator. The flow diagram for the entire extraction process is given below :



Scheme 3.1 : Extraction of Powdered leaves of *Tylophora indica* with various solvents

3.4 : Examination of the crude Ethyl acetate extract (Mass R_{EA}, 95 g)

The greenish gummy mass R_{EA} (95 g) of ethyl acetate extract when examined for solubility, it was found soluble in ethyl acetate and methanol. When TLC was done on silica gel plates in different solvent systems and viewed under UV light and exposed to iodine vapor no clear resolution was obtained giving the impression that the extract might

be a mixture of several components. The total extract was then dissolved in Ethyl acetate (750 ml) and filtered by Whatman no.1 filter paper to remove any suspended impurities and methanol was gradually added to it until the solution became turbid and the resultant turbid solution was allowed to stand at room temperature for several hours to observe any crystallization.But no such crystallization occured. On further addition of methanol a gummy mass was precipitated. The supernatent from the gummy mass was separated by centrifugation. The supernatent on evaporation and drying gave a greenish oily mass S_{EA}(73 g). The residue from centrifugation on drying also gave another greenish gummy mass (~ 16 g) which was kept aside.

The fraction S_{EA} was found to be soluble in ethyl acetate and methanol.The TLC behavior of its ethyl acetate solution was examined in several solvent systems. But no resolution was found with any of the solvent system. However, the best resolution was observed in the solvent system n-hexane : Ethyl acetate (1:4) which showed two spots at R_f 0.58 and 0.46 with long tailing extending over the entire plate. So, it was decided to have an attempt for column chromatographic separation of the fraction.

3. 5 : Column chromatographic fractionation of the fraction S_{EA}:

The fraction S_{EA} (7.5 g) was dissolved in minimum volume of Ethyl acetate and was adsorbed in silica gel (~ 15 g).The solvent from the adsorbed mass was removed by rotary evaporation under reduced pressure. It was then carefully poured on the top of a column (49×3 cm) made of silica gel with the solvent n-hexane. The column was eluted first with n-hexane followed by mixtures of solvents of n-hexane - Ethyl acetate and Ethyl acetate-Methanol. The eluents were collected in ~7 ml portions in test tubes and were examined on TLC plates.

Table 3.1 : Column chromatographic fractionation of the fraction S_{EA}

Collection Nos.	TLC examination and observation	Inference	Yield	Fraction Nos.
1-2	One single spot R_f 0.69 (100% n-hexane)	Might be one pure compound	5 mg	S_{EA-1}
3-14	One spot R_f 0.69 with long tailing from base line (100% n-hexane)	Might be a mixture of compounds	350 mg	S_{EA-2}
15-36	Tailing from base line (2% EtOAc in n-hexane)	No resolution. May be a mixture of few compounds	40 mg	S_{EA-3}
37-46	One spot, R_f 0.5 and tailing in base line (20% EtOAc in n-hexane). Vaneline H_2SO_4 spray, iodine inactive	Might be a single compound with slight impurities	300 mg	S_{EA-4}
47-59	Mixtures of three compounds at R_f 0.82; 0.52 and 0.25 with impurities (0.5% n-hexane +9% $CHCl_3$ +0.5% EA)	Might be a mixture of three components with impurities	1.10 g	S_{EA-5}
60-70	Three spots R_f 0.77; 0.5 and 0.15 with tailing at base line (0.5% n-hexane + 9% $CHCl_3$ + 0.5% EtOAc)	Might be a mixture of three compounds with impurities.	1.33 g	S_{EA-6}
71-90	Four spots, R_f 0.8; 0.52; 0.42 and 0.17 with tailing at base line (0.5% n-hexane + 9% $CHCl_3$ + 0.5% EtOAc)	Might be a mixture of four compounds with impurities	400 mg	S_{EA-7}
91-126	Four spots, R_f 0.8; 0.55; 0.45 and 0.2 with tailing from base line (0.5 % hexane + 9% $CHCl_3$ + 0.5% EtOAc)	Might be a mixture of four compounds with impurities	450 mg	S_{EA-8}
127-144	Tailing (30% EtOAc in n-hexane	No resolution	200 mg	S_{EA-9}
145-200	Tailing (40% EtOAc in n-hexane)	No resolution	500 mg	S_{EA-10}

3.5.1 : Study on the Fraction S_{EA-1} :

The fraction S_{EA-1} (5 mg) was a colorless liquid compound. It was soluble in n-hexane and Chloroform. It gave one single spot at R_f 0.69 in 100% n-hexane. It is a pure compound and was named as EA-1. The amount of the sample being very small further studies on the fraction was not possible.

3.5.2 : Study on the Fraction S_{EA-2} :

The fraction S_{EA-2} (350 mg) was a light orange colored liquid compound. It was soluble in n-hexane and chloroform. It gave one spot at R_f 0.69 with long tailing from base line in 100% n-hexane. The resolution of this fraction was not so good. Also the amount of this fraction being very small, it was not processed any further.

3.5.3 : Study on the Fraction S_{EA-3} :

The fraction S_{EA-3} (40 mg) was a deep orange colored liquid. It was soluble in n-hexane and chloroform. When subjected to TLC on silica gel plate using several solvent systems, no good resolution was obtained. The amount of the fraction being small and also the resolution in TLC being very poor, further studies on this fraction was not continued.

3.5.4 : Study on Fraction S_{EA-4} :

Fraction S_{EA-4} (300 mg) was a greenish solid mass and was soluble in n-hexane, Chloroform, Dicholoromethane and Ethyl acetate. When TLC of the mass was done on silica gel plate with various solvent systems and viewed under UV light and exposed to iodine vapor no spot was seen. But when the plates were sprayed with Vaniline-sulphuric acid spray and heated in an oven at 105°C for a few minutes, two major spots with tailing were observed at R_f 0.5 and 0.27. Therefore, the fraction was subjected to column chromatographic separation.

3.5.4.a : Column chromatographic separation of fraction S_{EA-4} :.

The fraction S_{EA-4} (300 mg) was dissolved in minimum volume of n-hexane and adsorbed in an appropriate amount of silica gel and the solvent from the adsorbed mass

was removed by rotary evaporation under reduced pressure. The dried adsorbed material was then poured very carefully on the top of a column made on silica gel using n-hexane as the solvent. The column was eluted successively with n-hexane, mixtures of n-hexane-Chloroform and ethyl acetate. The eluents were collected in test tubes in 4 ml portions and 35 such tubes were collected. The eluent fractions were monitored by TLC on silica gel plates developed by Vaniline-sulphuric acid spray. The similar fractions were pooled together. Four such fractions were obtained whose characteristics have been described in table 3.2.

Table 3.2 : Column chromatographic separation of fraction S_{EA-4}.

Collection Nos.	TLC examination and observation	Inference	Yield	Fraction nos.
1-5	-	-	-	$S_{EA-4(a)}$
6-10	A single spot with a very minor tailing, R_f 0.5(20% EtOAc in n-hexane)	Might be one compound with minor impurities	80mg	$S_{EA-4(b)}$
11-20	One spot with tailing , R_f 0.27 with very poor resolution (20% EtOAc in n-hexane)	Might be one compound with major purities	20 mg	$S_{EA-4(c)}$
21-35	No good resolution	Might be impurities	10 mg	$S_{EA-4(d)}$

3.5.4.b : Study on the fraction $S_{EA-4(b)}$:

The fraction $S_{EA-4(b)}$ (80 mg) was soluble in the solvents n-hexane Chloroform and Ethyl acetate. When subjected to TLC on a silica-gel plate using the solvent system n-hexane : EtOAc (80 :20) developed by Vaniline-sulphuric acid spray, a single spot was found at R_f 0.5 with a minor tailing in the upward direction. So, the fraction was subjected to repeated crystallization using the solvent Chlorform with a trace amount of n-hexane

until a single spot was obtained on silica gel TLC plate developed by Vaniline- sulphuric acid spray. The amount of the recrystalized pure compound was 20 mg. It was named as EA-4 for simplicity in forthcoming examiunations.

3.5.5 : Studies on the fractions S_{EA-5} and fraction S_{EA-6} :

The fraction S_{EA-5} (1.10 g) was a green colored fraction. It was soluble in solvents n-hexane, Chloroform and Ethyl acetate. Fraction S_{EA-6} (1.33 gm) was also a green colored solid. It was also soluble in n-hexane, Chloroform and Ethyl acetate. When the fraction S_{EA-5} was subjected to TLC on a silica gel plate using the solvent system n-hexane : $CHCl_3$: EtOAc (5 : 90 : 5) and developed in iodine vapor, three spots each one with tailing were observed at R_f 0.82, 0.52 and 0.25. Under identical conditions when the TLC spots of this fraction S_{EA-5} was compared with those of S_{EA-6} (1.33 g), these were found similar but the resolution in TLC was found much better in case of the fraction S_{EA-6}. Therefore, the two fractions were considered as the same but the resolution being better in case of S_{EA-6}, further studies were continued on fraction S_{EA-6} instead of fraction S_{EA-5}.

3.5.5.a : Column chromatographic separation of fraction S_{EA-6}:

The whole of the fraction S_{EA-6} (1.33 g) was dissolved in minimum volume of chloroform and adsorbed in silica gel (~ 2.8 g). The dried adsorbed mass was poured on the top of a silica gel column (dimension) made in Chloroform. The solvents used for the successive elution of the column were (a) chloroform, (b) mixture of n-hexane, Chloroform and Ethyl acetate and (c) Ethyl acetate and Methanol. In all, 150 tubes were collected, each tube contained (~6 ml). These were monitored by TLC. The collections were combined separately on the basis of their TLC behavior. The results of the chromatographic separation is given in Table- 3.3

Table-3.3: Column chromatographic separation of fraction S_{EA-6}

Collection Nos.	TLC examination and observation	Inference	Yield	Fraction nos.
1-21	Tailing from base line (50% n-hexane in chloroform)	No resolution	20 mg	S_{EA-6a}
22-29	Two spots R_f 0.47 and 0.37 with some impurities (80% $CHCl_3$ in n-hexane)	Mixture of at least two compounds	200 mg	S_{EA-6b}
30-49	Two sports R_f 0.40 and 0.22 with impurities at base line (100% $CHCl_3$)	A mixture of minimum two compounds	280 mg	S_{EA-6c}
50-134	One spot with long tailing R_f 0.29 (5% EtOAc in n-hexane)	Might be a single compound with impurities	130 mg	S_{EA-6d}
135-140	Tailing	No resolution	50 mg	S_{EA-6e}

3.5.5.b : Study on the fraction S_{EA-6}:

Fraction S_{EA-6c} (280 mg) was appeared to be a white crystalline compound. It was soluble in Chloroform and Ethyl acetate. But TLC examination of this fraction on silica gel plate showed one major spot with tailing having R_f at 0.22 (100% $CHCl_3$) along with some impurities at the base line. This fraction was recrystallised three times from the solvent chloroform with trace amount of n-hexane. The recrystallized product was given the name S_{EA-6c3} (80 mg). On TLC examination this recrystallized product gave a single spot in solvent system Chloroform : Ethyl acetate (99 : 1) with some impurities in the base line. So, it was subjected to column chromatographic analysis again.

3.5.5.c : Column chromatographic purification of $S_{EA\text{-}6c3}$ (80 mg) :

The impure fraction $S_{EA\text{-}6c3}$ (80 mg) was dissolved in chloroform and adsorbed in minimum quantity of silica gel and dried completely under reduced pressure. The adsorbed mass was then chromatographed over a small column of silica gel made in chloroform. Eluting solvents were chloroform and ethyl acetate. The elunets were collected in test tubes in 4 ml portions and were examined by TLC. The eluents which showed similar TLC behavior were pooled together. The results of the column chromatographic separation is given in the Table-3.4.

Table-3.4: Column chromatographic separation of $S_{EA\text{-}6c3}$ (80 mg)

Collection Nos.	TLC examination and observation	Inference	Yield	Fraction nos.
1-5	A single spot, R_f 0.25 (1% EtOAc in Chloroform).	Might be one compound	45 mg	$S_{EA\text{-}6c3(a)}$
6-10	Tailing(1% EtOAc in Chloroform)	Might be a mixture of two compounds	10 mg	$S_{EA\text{-}6c3(b)}$

3.5.5.d : Study on the Fraction $S_{EA\text{-}6c3(a)}$

The fraction $S_{EA\text{-}6c3(a)}$ (Table-3.4 , 45 mg) obtained from column chromatographic separation was a white waxy compound and was soluble in chloroform and Ethyl acetate. On subjection to TLC examination in the solvent system Ethyl acetate : chloroform (1 : 99), a single spot was foundat R_f 0.25. So, this fraction was considered as a pure compound and was designated as EA-2 for simplicity.

3.5.5.e : Study on the fraction S_{EA-6d}:

Fraction S_{EA-6d} (130 mg) was a greenish solid mass. It was soluble in n-hexane and chloroform. TLC examination of this fraction on silica gel plate showed one spot with long tailing (R_f 0.3) in the solvent system n-hexane : $CHCl_3$: EtOAc (8 : 1 : 1).

3.5.5.f : Column chromatographic separation of Fraction S_{EA-6d}.

Fraction s_{EA-6d} (130 mg) was dissolved in minimum volume of n-hexane and adsorbed in an appropriate quantity of silica gel. The solvent from the adsorbed mass was totally removed by rotary evaporation under reduced pressure. The adsorbed mass was then placed very carefully on the top of a column made of silica gel in n-hexane. The column was eluted successively with n-hexane and mixture of n-hexane, chloroform and ethyl acetate. The eluents were collected in 50 test tubes, each tube containing (~ 4 ml). The fractions were monitored by TLC and the similar fractions were pooled together. Pooled fractions were three whose characteristics are given in the table –3.5.

Table –3.5: Column chromatographic separation of fraction S_{EA-6d}.

Collection Nos.	TLC examination and observation	Inference	Yield	Fraction nos.
1-11	-	-	-	$S_{EA-6d(1)}$
12-20	A single spot with a very minor tailing, R_f 0.62(5% EtOAc in chloroform)	Might be one compound	75mg	$S_{EA-6d(2)}$
21-30	One spot, R_f 0.62 with tailing (5% EtOAc in chloroform)	Might be one compound with impurities	10 mg	$S_{EA-6d(3)}$
31-50	No good resolution	Might be impurities	5 mg	$S_{EA-6d(4)}$

3.5.5.g : Study on the Fraction $S_{EA\text{-}6d(2)}$:

The fraction $S_{EA\text{-}6d(2)}$(Table 3.5, 75 mg) was highly soluble in n-hexane and $CHCl_3$ and also solube in EtOAc. When subjected to TLC in silica gel plate using solvent system $CHCl_3$: EtOAc (95 : 5), it showed single spot at R_f 0.62 but still with very very minor tailing. It was finally purified by repeated recrystallization from the solvent chloroform with a trace of n-hexane. The pure product was waxy white and its amount was 60 mg. For simplicity it was given the name EA-3.

3.5.6 : Study on the Fraction $S_{EA\text{-}7}$:

The fraction $S_{EA\text{-}7}$ (400 mg) was a deep green colored fraction. It was soluble in Chloroform and Ethyl acetate. When subjected to TLC on a silica-gel plate using the solvent system $CHCl_3$: EtOAc : n-hexane(90:5:5) and exposed to iodine vapor, it showed at least four spots each one with tailing above and below at R_f 0.8; 0.52; 0.42 and 0.17 respectively. When attempted for column chromatographic separation no good result was obtained and hence no further studies were possible on this fraction.

3.5.7 :Study on the Fraction $S_{EA\text{-}8}$:

The fraction $S_{EA\text{-}8}$ (450 mg) was a deep green colored fraction. It was soluble in Chloroform, Ethyl acetate and Methanol. When subjected to TLC on a silica-gel plate using the solvent system $CHCl_3$: EtOAc : n-hexane (90 : 5 : 5) and exposed to iodine vapor several closely related spots with tailing were found. Attempt for column chromatographic separation was not successful. Eventually further studies were not possible on this fraction.

3.5.8 : Study on the Fraction $S_{EA\text{-}9}$:

The fraction $S_{EA\text{-}9}$ (200 mg) like the fraction $S_{EA\text{-}8}$ was also deep green colored fraction. It was soluble in Chloroform, Ethyl acetate and Methanol. When subjected to TLC on a silica-gel plate using the solvent system EtOAc : n-hexane (30 : 70) and developed in iodine vapor, a continuous line from the base to solvent front was observed. Hence no further study was continued on this fraction.

3.5.9 :Study on the Fraction S_{EA-10}:

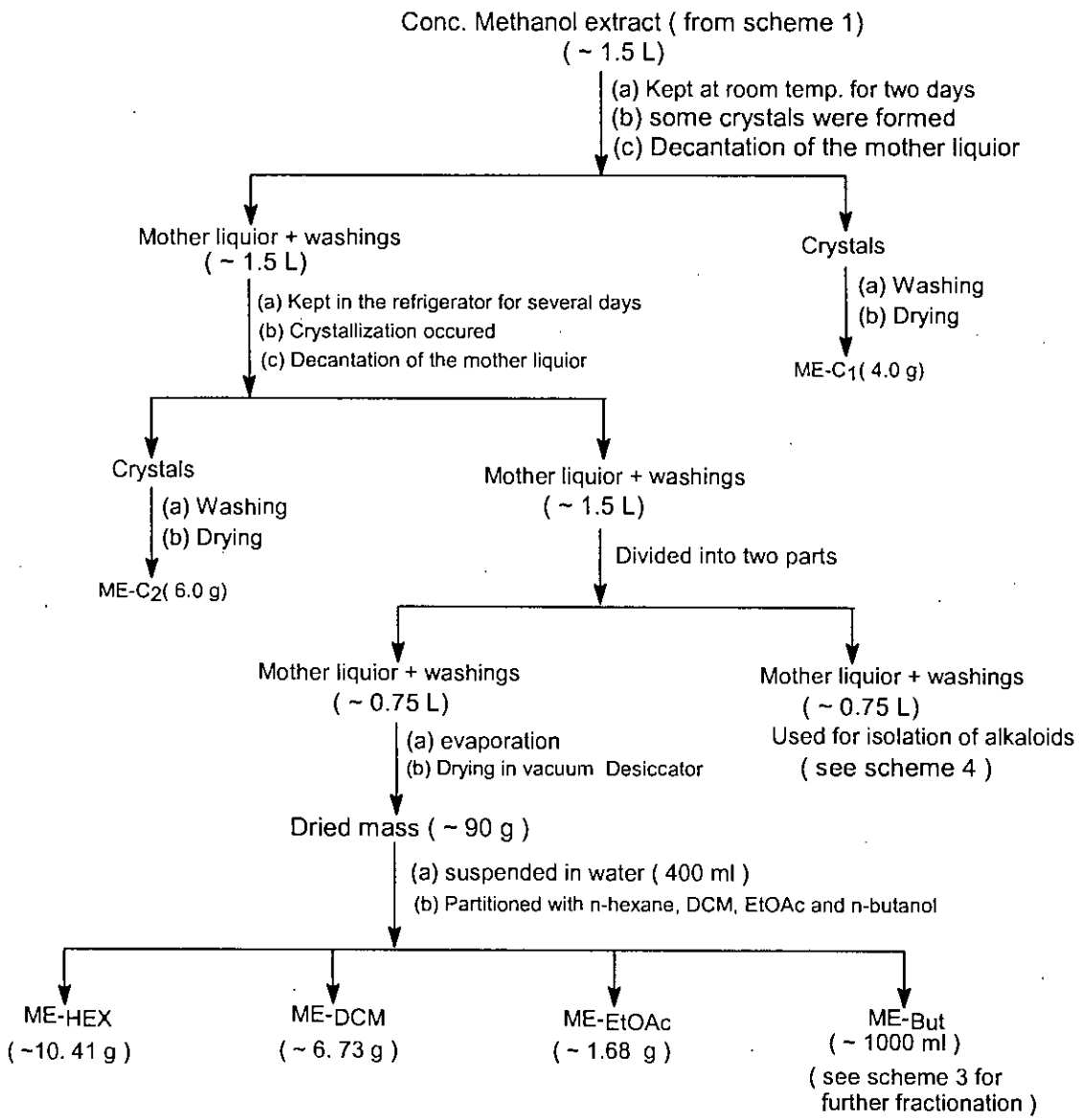
The fraction S_{EA-10} (500 mg) was deep green gummy fraction. It was soluble in Chloroform , Ethyl acetate and Methanol. When subjected to TLC on a silica-gel plate using the solvent system EtOAc : n-hexane (40 : 60), like the fraction S_{EA-9}, it also showed a continuous line from the base line to the solvent front. So, further studies on this fraction was not possible.

3.6 : Fractionation of the crude methanol extract :

The whole of the methanol extract when evaporated under reduced pressure to a volume of about 1.5 L some fine crystals were visible on the walls of the evaporating vessel. So, further evaporation was stopped and the concentrated ME-extract (1.5 L) was transferred from the evaporating flask to a 2L conical flask and was allowed to stand at room temperature for 2 days when some crystals were formed at the bottom and on the walls of the flask. The mother liquior was decanted and the crystals were at once washed with a little amount of cold methanol. The washings were added to the decanted mother liquor. The crystals were dried in a vacuum desiccator over P_2O_5 yielding the fraction ME-C_1 (4.0 g). An amount of 100 ml from the decanted mother liquior and washings (~ 1.6 L) was taken out and evaporated to dryness in a rotary evaporator under reduced pressure and finally dried in a vacuum desiccator over P_2O_5 yielding an amount of ~12 g. So, the total ME extract was [~ (16 X 12 + 4) = ~ 196 g]. The dried 12 g ME extract was again dissolved in about 100 ml methanol and added to the mother liquior. The total mother liquior (~1.5 L) was then allowed to stand in the refrigerator for several days when another crop of crystals were formed. The decantation of the mother liquior followed by washing and drying of the crystals yielded the 2nd crystalline fraction ME-C_2 (6 g).

50 % of the decanted mother liquior was dried by rotary evaporation under reduced pressure to a deep green mass (~ 90 g). The green mass was suspended in water (~ 400 ml) and was partitioned by solvents in order of their increasing polarity e.g. n-hexane, Dichloromethane, Ethyl acetate and n-butanol. The n-hexane, Dichloromethane and Ethyl

acetate partioned fractions on drying yielded the products $ME\text{-}_{HEX}$ (10.41 g), $ME\text{-}_{DCM}$ (6.73 g) and $ME\text{-}_{EtOAc}$ (1.68) respectively. The process of the fractionation is shown in the scheme 3.2.



Scheme 3.2. Fractionation of the crude methanol extract

3.6.1 : Study on n-hexane partitioned product (ME-$_{HEX}$) :

The fraction ME-$_{HEX}$ (10.41 g) was a deep green gummy mass. It was soluble in n-hexane and Chloroform. When TLC of this fraction was done on silica gel plate with the solvent system EtOAc : n-hexane (40 : 60) so many spots were found at R_f

With long tailing from base line. The resolution between the spots were not good. So further studies on this fraction was not continued.

3.6.2 : Study on Dichloromethane partitioned product (ME-$_{DCM}$) :

The fraction ME-$_{DCM}$ (6.73 g) was a deep green gummy mass. It was soluble in Dichloromethane and Chloroform. When carried out TLC of this fraction on silica gel plate with the solvent system EtOAc : n-hexane (40 : 60) so many spots were found at R_f With long tailing from base line. The resolution between the spots were not good. So further studies on this fraction was not done.

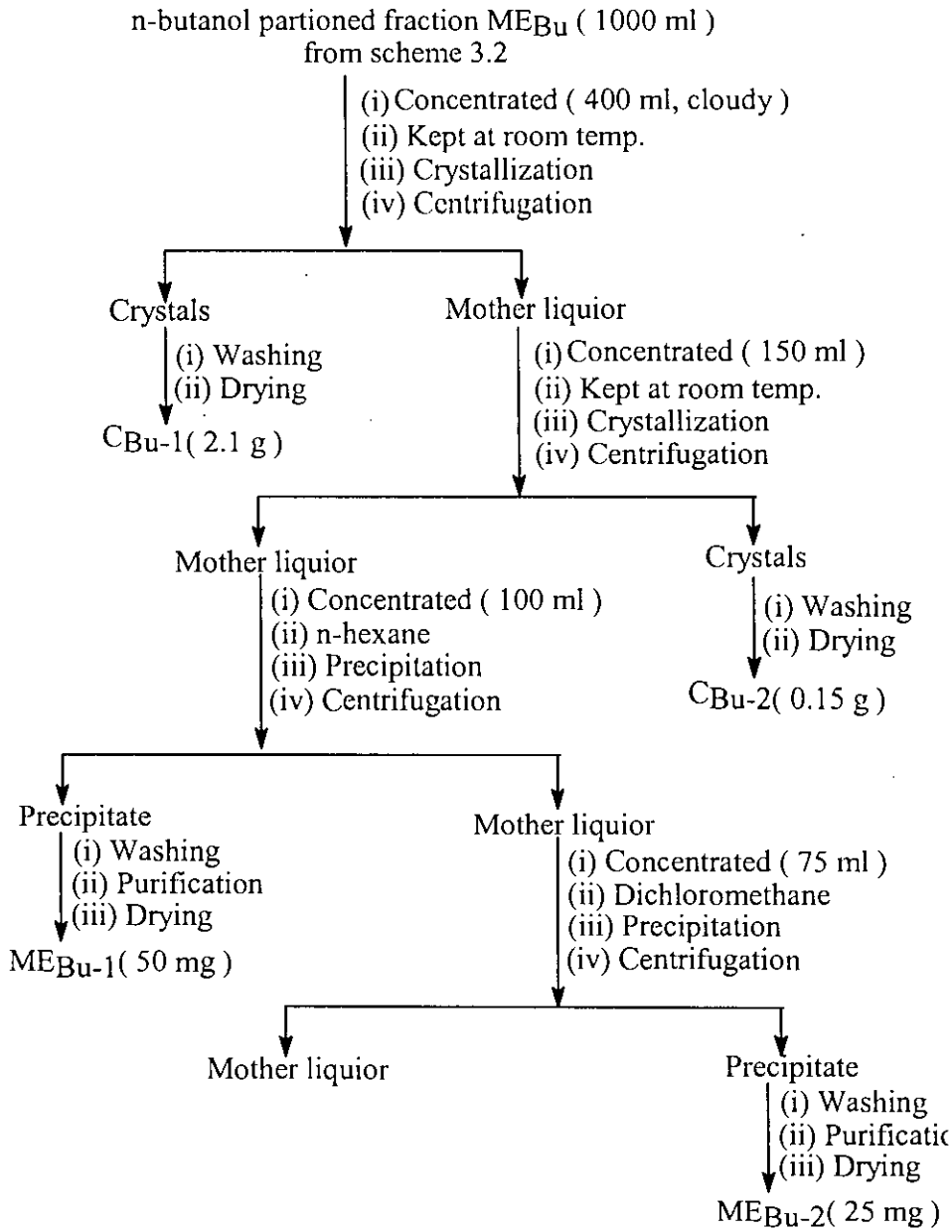
3.6.3 : Study on EtOAc partitioned product (ME-$_{EtOAC}$):

The fraction ME-$_{EtOAC}$ (1.68 g) was a greenish colored mass. It was soluble in Chloroform and Ethyl acetate. When subjected to TLC of this fraction on silica gel plate using the solvent system n-hexane : DCM : EtOAc (20 : 20 : 60) some resolution was observed. On the basis of this TLC a column chromatographic separation was done but no good fraction was found. Then further studies on this fraction were not continued.

3.6.4 : Study on n-butanol partitioned product (ME-$_{Bu}$)

The fraction obtained on partitioned with n-butanol (1000 ml) on concentration (400 ml) became turbid which on standing at room temperature formed crystals. The crystals were separated by centrifugation. The crystals from centrifugation was dried in a vacuum desiccator to yield the fraction C-$_{Bu1}$ (2.1 g). The mother liquior from centrifugation on further concentration (150 ml) became turbid again and was allowed to stand at room temerature for several hours while a 2nd crop of crystals were formed. These were also separated by centrifugation. The crystalline centrifugate on drying yielded the fraction C-$_{Bu2}$ (0.15 g). The mother liquior from centrifugation was further concentrated (100 ml)

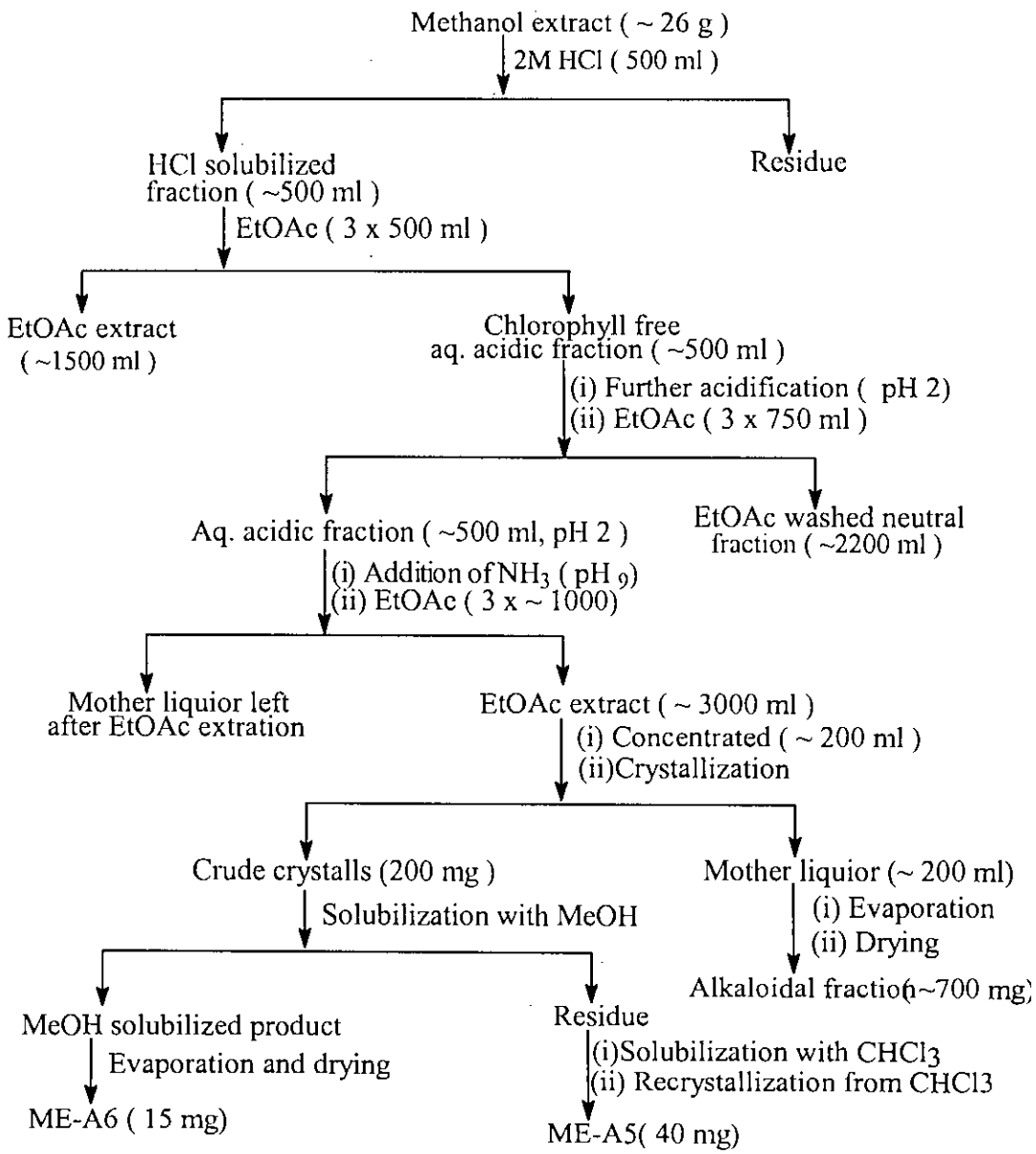
and was kept at room temperature for several days with no crystallization and hence n-hexane was added until the entire solution became cloudy. The cloudy solution was allowed to stand overnight at room temperature while a batch of crystals were formed. The crystals were removed by centrigugation, washed with n-hexane and dried in a vacuum desiccator using P_2O_5 as the desiccating agent to give an yield of 200 mg. This on several times recrystallization from methanol followed by addition of n-hexane yelded the compound ME-$_{BU1}$ (50 mg). The mother liquoir and the washings from (ME-$_{BU1}$) was further concentrated (75 ml) and kept at room temperature for several days with no crystallization even with addition of n-hexane. So, n-hexane was removed from this fraction by rotary evaporation. Instead of n-hexane when a small amount of dichloromethane was added, the solution became cloudy and this cloudy solution on standing at room temperature for about 24 hours afforded another batch of crystalline product. The crystals were separated by centrifugation and was washed with cold DCM for several times. The DCM washed crystals was dissoved in methanol and repeatedly recrystallized for several times with addition small amounts of dichloromethane finally yielded ME-$_{BU2}$ (25 mg).



Scheme 3.3 : Fractionation of n-butanol partioned fraction from Methanol extract

3.7 : Acid-base seperation of the methanol extract for isolation of alkaloids :

~200 ml from the remaining 50% of the original mother liquior was evaporated and dried in a vacuum desiccator to give a greenish mass (~ 26 g) and 2M HCl was added to it when a part of it solubilized. 2M HCl soluble portion (~ 500 ml) was decanted and extracted with EtOAc (3 X 500 ml) to remove chlorophyll. The aqueous acidic solution was further acidified with 2M HCl until the pH of the solution became 2. The solution was thoroughly washed with EtOAc (750 ml X 3) to remove the neutral components. The Ethyl aceate extract (~2200 ml) was evaporated and dried to give to give a mass (200 mg). The Ethyl acetate extract free solution was basified with 30 % ammonia solution (~ 500 ml) until the pH became 9. The basified solution was thoroughly extracted with Ethyl acetate (~1000 ml X 3). The Ethyl acetate extract (~ 3000 ml) was concentrated (~ 200 ml) when some crystals were appeared on the walls of evaporating flask. The entire concentrated solution was allowed to stand at room temperature for about several hours whereby some crystals were formed. The crystals were separated from the mother liquior by decantation. A portion of the crystals went into solution in methanol which when evaporated and dried gave a fraction ME-A6 (15 mg). The remainder of the crystals were soluble in chloroform.(120 mg) which on several times recrystallization from chloroform with trace amount of n-hexane yielded the fraction ME-A_5 (40 mg). The mother liquior which was decanted from the crystals on evaporation and drying gave the alkaloidal fraction (700 mg). Further studies on this fraction are in progress.



Scheme 3.4 : Fractionation of the MeOH extract for isolation of alkaloids

3.8 : Fractionation of the aquous part remained after partition:

The aqueous part remained after partition was evaporated under reduced pressure and finally dried in a vacuum desiccator yielding the fraction Me $_{BuH}$ (4 g). Methanol (100 ml) was added to it whereby a portion of it went into solution. The solution was separated from the remainder by centrifugation. The residue from the centrifugation was dried (2.78 g). The solution from centrifugation was concentrated (50 ml) and DCM was added to it until the solution became cloudy. It was allowed to stand at the room temperature for several hours whereby some yellowish crystals were formed. The mother liquior from the crystals were separated by decantation and was washed with cold dichloromethane and EtOAc. This was recrystallized several times from MeOH to give the fraction ME-$_{BUH1}$(85 mg). The mother liquior was concentrated and tried for precipitation with DCM and n-hexane with no result and hence the solvent was removed and dried in a vacuum desiccator to give the fraction ME-$_{BUH2}$ (220 mg).

3.9.1 : Properties of the isolated compound EA-1 (S_{EA-1}):

Physical appearance: Colourless liquid compound

Solubility: Soluble in n-hexane and chloroform.

TLC: Single spot with R_f 0.69 in 100% n-hexane.

Amount: 5 mg

The amount of the sample was so small. So further analysis was not possible.

3.9.2 : Properties of the isolated compound EA-2 ($S_{EA-6C3(a)}$)

Physieal appearance: White solid compound.

Solubility: Soluble in chloroform and ethyl acetate.

Melting Point: The compound melts at 110°C-112°C

TLC: Single spot with R_f 0.25 in a solvent system EtOAc: $CHCl_3$ (1:99)

Amount: 45 mg

IR Spectra of EA-2 in KBr :

ν_{max} 3450 (OH), 2960, 2850 (-C-H), 1450, 1400 (C-H bending), 1050,960, 810 cm^{-1}

^{1}H-nmr spectral data of compound EA-2

δ 0.699 (s) , 1.009(s)	2 x CH_3
0.68, 0.82	2 x CH_3
0.807 (t)	1 x CH_3
1.025 (d)	1 x CH_3
3.4 (ddq)	
5.04 (dd) Trans olefinic protons	
5.17 (dd) ,,	
5.35 (br.d) olefinic proton at C-6	
1.5 (m), 1.82 (m) and 2.28 (m)	

^{13}C-nmr spectral data of EA-2

37.24 (C-1), 31.65 (C-2), 71.80(C-3), 42.31(C-4), 140.73 (C-5), 121.70 (C-6),31.90(C-7), 31.87(C-8), 50.15 (C-9), 56.50 (C-10), 21.10 (C-11), 39.77(C-12), 42.31(C-13), 56.86(C-14), 24.35(C-15), 29.15(C-16), 56.10(C-17),12.04 (C-18), 19.40 (C-19), 40.47 (C-20), 21.10 (C-21), 138.30(C-22),129.27 (C-23), 51.23 (C-24), 31.90 C-25), 21.20(C-26), 19.02 (C-27), 25.40 (C-28), 12.24(C-29)

3.9.3 : Properties of the isolated compound EA-$_3$ ($S_{EA\text{-}6d(2)}$)

Physical appearance: White waxy type compound.

Solubility: Highly soluble in n-hexane and chloroform and also soluble in ethyl acetate.

Melting Point: The compound melts at 53°C-54°C

TLC: Single spot with R_f 0.62 in a solvent system $CHCl_3$: EtOAc (95:5)

Amount: 60mg

IR Spectra of EA -3 in KBr :

ν_{max} 3500 (O-H str.), 2919, 2849 (C-H str.), 1733, 1700(C=O str.), 1640 (-C=C-str.), 1450 (C-H bend), 1300, 920 (C-O), 750cm^{-1}

^{1}H- nmr spectral data of EA -3

5.32 (m, 1H, =CH), 2.33-2.36 (t, 4H, 2 x CH_2), 2.03 (m, 1H), 1.60-1.66 (m, 4H, 4 x -CH), 1.25 (m, 41H, 2 x CH_3 , 17 x CH_2, 1 x OH), 0.8 (t, 6H, 2 X CH_3)

^{13}C- nmr spectral data of EA -3

190.60, 180.10, 130.00, 127.20, 112.86, 65.40, 34.00, 31.90, 29.76, 29.66, 29.64, 29.63, 29.60, 29.42, 29.36, 29.35, 29.20, 29.13, 29.05, 29.02, 27.19, 27.17, 24.70, 24.67, 24.58, 22.70, 22.14, 21.70, 14.10

Mass fragmentation of EA-3

Considering molecular ion peak at m/z at 511, The m/z 510 = [M^+ - H] , The m/z 425= [M^+ - H - C_6H_{13}], thc m/z 340 = [M^+ - H - C_6H_{13} - C_6H_{13}], The m/z 284 (base peak) = [M^+ - H - C_6H_{13} - C_6H_{13} - C_4H_8]

3.9.4 : Properties of the isolated compound EA-4 ($S_{EA-4(b)}$):

Physical appearance: White powder type compound.

Solubility: Soluble in the n-hexane, chloroform and also ethyl acetate.

Melting Point: The compound melts at 70°C-71°C

TLC: Single spot with R_f 0.5 in a solvent system n-hexane: EtOAc (80:20)

Amount: 20 mg

IR Spectra of EA-4 in KBr :

ν_{max} 3300 (O-H), 2917, 2849 (C-H str.), 1700(C=O str.), 1630 (-C=C-str.), 1460, 1445 (C-H bend), 1280 (C-N str.) cm^{-1}

^{1}H- nmr spectral data of EA-4

2.35 (t, 2H, 1 X CH_2), 1.63 (m, 2H, 1 x CH)1.25 (m, 40H, 19 x CH_2, 1 x CH, 1 x OH), 0.88 (t, 3H, 1 x Me)

^{13}C- nmr spectral data of EA-4

196.35, 170.60, 112.80,100.37, 65.40, 40.00, 31.90, 30.30, 29.70 (2 carbons), 29.67 (2 carbons), 29.65, 29.60, 29.36, 29.30 (4 carbons), 29.20 (2 carbons), 27.00, 26.20 (2 carbons), 24.70, 22.70, 14.11

Mass fragmentation of EA-4

MS= M^+(429), 412 [M^+–OH], 355[M^+–OH – C_4H_9, the m/z 355 is the base peak], 341 [M^+ –OH – C_4H_9 – CH_2,]

3.9.5 : Properties of the isolated compound Me-A-5:

Physical appearance: Deep orange powder type compound.

Solubility: Soluble in Chloroform, ethyl acetate and methanol

Melting Point: The melting point of the sample above 178- 180°C.

TLC: Single spot at R_f 0.65 in a solvent system MeOH : EtoAc : $CHCl_3$ (50 : 40 :10)

IR Spectra of A_5 in KBr :

ν_{max} 3300 (br, N-H str.) 3010 (=C-H str.), 2920, 2840 (-CH str.), 1616, 1512 (C=C, Aro),1467,1442,1425 (CH,bend), 1195,1147 (C-N), 1035,1016 (C-O), 842 cm^{-1}

^{1}H-nmr spectral data of A_5 :

7.75 (s, 2H), 7.22 (s, 1H), 7.06 (s, 1H), 4.53 (d, 1H), 4.08 (s, 6H, 2 x OMe), 4.01 (s, 3H, 1 x OMe), 3.99 (s, 3H, 1 x OMe), 3.91 (septet, 1H), 3.82 (d, 1H), 3.59 (dd, 1H), 3.43 (d, 1H), 3.27 (dd, 1H), 2.85 (dd, 1H), 2.42 (m, 2H), 2.20 [m(br), 2H] , 2.08 (m, 1H), 1.90 (m,H),1,75 (m, 1H) ,1.24 [s, 6H, 2 x CH_3], 0.85 (m, 2H)

^{13}C-nmr spectral data of A_5 :

148.65, 148.46, 148.38 (C-2,3,6,7), 126.18, 125.73, 124.23, 123.57, 123.38 (C-1a, 4a, 5a, 8a, 9a,14a) 103.93, 103.39, 103.27,103.07(C-1,4, 5,8), 60.19 (C-14), 56.01 55.88, 55.84 (4 x OMe),56.47 (C-9), 55.70 (C-13), 55.45 (C-19), 55.07 (C-13a), 32.56 (C-11), 31.06 (C-16), 30.72 (C-18), 29.68 (C-12), 22.95 (C-17),14.10 (2 xCH_3).

^{13}C-Dept spectral data of A_5 :

Upside signals : 105.28, 104.73, 104.62, 104.43 [C-1,4,5,8 (4 x CH)], 61.53 (C-14, 1 x OCH) , 57.37, 57.25, 57.21, 57.09 [C-2,3,6,7, (4 x OCH_3)], 56.80 (C-13a, N x CH), 55.70 (C-13), 55.45 (C-19), 31.06 (C-12, 1 x CH) ,15.48 (2 x CH_3),

Downside signals : 56.47 (C-9, NCH_2), 32.56 (C-11, NCH_2), 31.06(C-16, NH-CH_2) 30.72 (C-18, CH_2), 22.95 (C-17)

^{1}H-^{1}H Cosy spectral data of A_5 :

4.53 (H-14)	↔	3.59 (H-13a)
3.91 (H-19)	↔	1.24 (H-methyl, 20,21)
3.59 (H-13a)	↔	3.27 (H-13)
3.27 (H-13)	↔	3.59(H-13a), 2.85(H-12), 2.42 (H-11)
3.82 (Ha–9)	↔	3.43 (H_b-9)
2.85 (H-12)	↔	3.27(H-13), 2.42(H-11)
2.42 (H-11a)	↔	2.85 (H-12), 3.43 (H_b-9)
2.20 (H_a-16)	↔	2.08 (H_b-16), 2.42 (H-11), 1.90 (H_a-18)
1.90 (H_a-18)	↔	2.08 (H_b-16), 2.42 (H-11)
0.85 (H-17)	↔	1.90(H_a-18), 2.20(H_a-16)

^{1}H-^{13}C Htetero-Cosy (HMBC) spectral data of A_5 :

7.75 (H-1, 8)	↔	103.93 (C-1), 103.39 (C-8)
7.22 (H-5)	↔	103.07 (C-5)
7.06 (H- 4)	↔	103.27 (C-4)
4.53 (H-14)	↔	60.19 (C-14), 148.38 (C-2)
4.08,4.01,3.99	↔	57.37, 57.25, 57.21, 57.09 (4 x OCH_3)
(4 x OCH_3 at C-2,3,6,7)		148.65, 148.46, 148.38 (C-2,3,6,7)
3.91(H-19)	↔	55.45 (C-19)
3.82(H_a-9)	↔	56.47 (C-9)
3.59 (H_a-13a)	↔	55.07 (C-13a)
3.43 (H_b-9)	↔	56. 47 (C-9)
3.27 (H_b-13)	↔	55.70 (C-13)
2.85 (H-12)	↔	29.68 (C-12)
2.42 (H-11)	↔	32.56 (C-11)
1.24 (H-20,21)	↔	14.10 (C-20,21)
0.85 (H-17)	↔	22.95 (C-17)

3.9.6: Properties of the isolated compound MeBu-1 :

Physical appearance: White powder type compound.

Solubility: Soluble in Methanol.

Melting Point: The melting point of the sample above 300°C.

TLC: Single spot at R_f 0.57 in a solvent system MeOH : EtOAc (90 : 10)

Amount: 50 mg

3.9.7 : Properties of the isolated compound MeBu-2

Physical appearance: Brown colored compound.

Solubility: Soluble in Methanol.

Melting Point: The melting point of the sample above 300°C.

TLC: Single spot at R_f 0.54 in a solvent system MeOH : EtOAc (90 : 10)

Amount: 25 mg

3.9.8 : Properties of the isolated compound MeBuH-1:

Physical appearance: Orange colored powder type compound.

Solubility: Soluble in Methanol.

Melting Point: The melting point of the sample above 300°C.

TLC: Single spot at R_f 0.62 in a solvent system MeOH : EtOAc (90 : 10)

Amount: 120 mg

3.9.9 : Properties of the isolated compound MeBuH-2:

Physical appearance: Orange crystal type compound.

Solubility: Soluble in Methanol.

Melting Point: The melting point of the sample above 300°C.

TLC: Single spot at R_f 0.60 in a solvent system 100% MeOH

Amount: 710 mg

4. 0 : CHAPTER 4

4.1 : Results and Discussion :

The various sections of this chapter 4 is a brief discussion of the work done on *T. indica* belonging to the family **Asclepiadaceae.**

4. 1.1 : Plant material :

From literature it appears that the aerial parts of the climbing perinnial herb *T. indica* Syn. *T. asthmatica* (Beng. Anthamul) and other species of the genera *Tylophora* under the family **Asclepiadaceae** are traditionally used against various ailments in Indo-Bangla subcontinent. It is reported that both the roots and leaves of this herb are an excellent substitute for ipecaucuanha. They have emetic, diaphoretic and expectorant properties. Its leaves were described as one of the best substitutes for ipecaucuanha and were recommended as useful in all cases indicating necessity of emesis and as a remedy for asthma, dysentery, catarrh and other affections in which ipecaucuanha is generally employed. A review on the phytochemical investigations on the herb revealed that along with other compounds, a good number of alkaloidal compounds [3,4,5,6] have been isolated from its roots and stems along with its leaves. But in comparison to the work on its stems and roots, little work has been done on its leaves. Since several alkaloids have already been isolated principally from its stems and roots, it is quite logical that its leaves should also contain alkaloids amongst other compounds. Alkaloids being physiologically active compounds, our major objective was to isolate, separate and purify alkaloidal compounds from the leaves of anthamul and to determine the molecular architecture of the isolated alkaloids along with other compounds isolated.

With the above aim in view, the herb was cultivated in sufficient quantities in the Club premises of the Bangladesh University of Engineering and Technology (BUET), Dhaka. Only the matured, fresh and sound leaves were collected for chemical analysis. These were cleaned, washed and dried in the shade and powdered (4.5 kg) for the purpose of the present work.

4.1.2 : Extraction of the plant powder :

The whole of the powder (4.5 kg) obtained from the leaves was extracted with various organic solvents and these were then subjected to fractionation according to the fractionation scheme-3.1 (section 3.3). The solvents from the fractions obtained from various extracts were evaporated by rotary evaporation and finally dried over phosphorus pentoxide (P_2O_5) in a vacuum desiccator under high vacuum.

4.1.3 : Isolation of the compounds EA-2, EA-3, EA-4 and A_5 :

The powder obtained from the grinding of dry *T.indica* leaves was successively extracted with the organic solvents petroleum ether, ethyl acetate and methanol (section 3.3). Ethyl acetate extract when subjected to fractionation and purification by applying various chromatographic and chemical methods yielded the three compounds EA-2, EA-3 and EA-4 amongst other products. Chemical and chromatographic fractionation of the methanol extract yielded compound A_5 along with some other compounds. The three fractions EA-2, EA-3 and EA-4 from ethyl acetate extract and fraction A_5 from methanol extract were pure and obtained in analyzable amounts and hence were subjected to further analyses by chemical and spectroscopic methods for identification and assignment of structures.

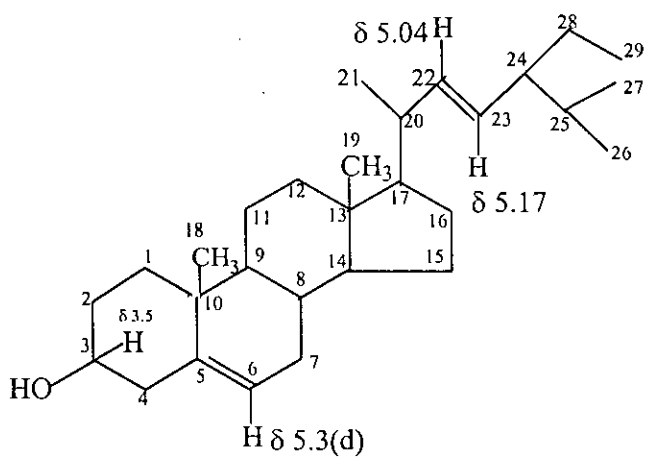
4.1.4 : Characterization of the compound EA-2 :

The compound EA-2 was a white solid, m.p 110-112°C. It was soluble in chloroform and ethyl acetate. When subjected to TLC in the solvent system EtOAc : $CHCl_3$ (1 : 99), it gave a single spot with R_f value 0.25. The compound vigorously responded to the Salkowski and Liebermann-Burchard color tests exhibiting its steroidal nature.

The IR spectrum Fig. 4.1 showed an absorption band at 3450 cm^{-1} indicative of a hydroxyl group (-OH). The sharp absorption bands at 2960 and 2850 cm^{-1} were demonstrative of aliphatic C-H stretching. The absorption bands at 1450, 1400 cm^{-1}

were indicative of $-CH_2-$ and $-CH_3-$ bending vibrations respectively [27]. The bends at 960 and 810 cm^{-1} were demonstrative of the steroidal nature of the compound [28].

The ^{1}H-nmr spectrum Fig. 4.2 (a, b) of the compound EA-2 showed two singlets (δ 0.699, 1.009, 1 x 2 CH_3) of 3H proton intensity each, two doublets (δ 0.68, 0.83, $2CH_3$), a 3H triplet (0.807, 1 x CH_3) and a 3H doublet (δ1.025, 1 X CH_3) typical for a steroidal type compound [Ref]. The doublet of quartet at δ3.50 is suggestive of of an oxymethine proton flanked by two methylene groups of cyclohexane ring system of a steroidal compounds. Its placement at the position C-3 of the ring A is supported by the biogenetic ground (Fig. 4.4) [29]. Two doublets of doublets (2dd, 1H each) at δ 5.04 and 5.17 are exhibitive of trans olefinic proton and an adjacent methane protons. A 1H broad doublet at δ 5.35 is indicative of an olefinic proton at C-6 [30]. With the help of the described IR and ^{1}H-nmr spectra, the following skeleton of a sterol can be drawn for the compound EA-2. The presence of the double bonds at C-5 and C-22 in this structure receive support from ^{13}C-nmr (δ 140.73 for C-5 and δ 121.70 for C-6 and δ 138.30 for C-22 and δ 129.27 for C-23) (Fig. 4.3 a,b,c and table 4.1).



The ^{13}C-nmr spectrum Fig.4.3 (a,b,c) of the compound EA-2 was analyzed and the chemical shifts (Table 4.1) of all the carbons were assigned on the basis of comparison with the reference structure[31]. The δ value for the C-3 carbon was at

71.80 was justified because it is attached to the oxygen atom of the hydroxyl group. The δ values for the carbon atoms C-5, C-6, C-22 and C-23 were 140.73, 121.70, 138.30 and 129.27 respectively are quite justified because of their olefinic nature and these values are also in agreement with the published data[31].

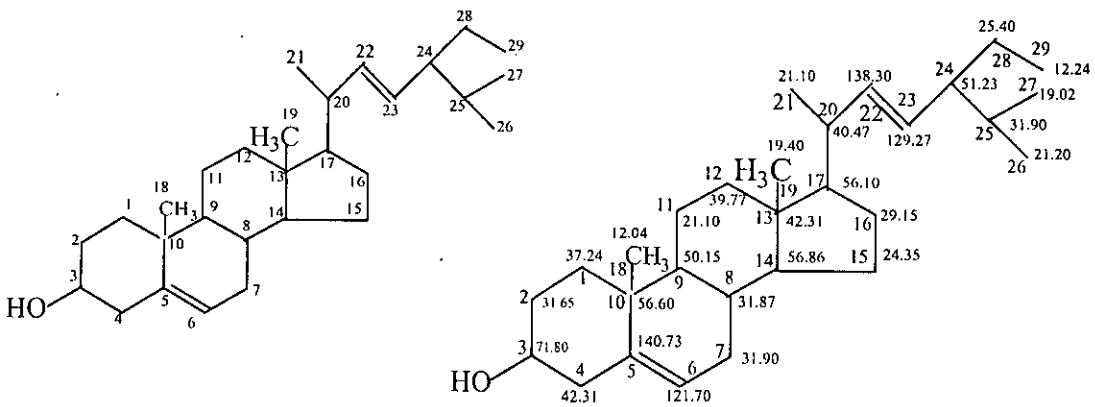
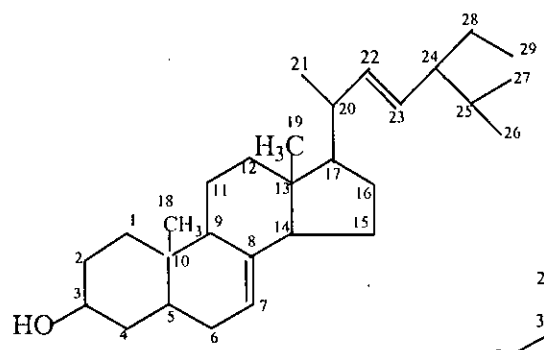


Table 4.1 : ^{13}C-nmr spectral data for the compound EA-2 compared with the published data [31]:

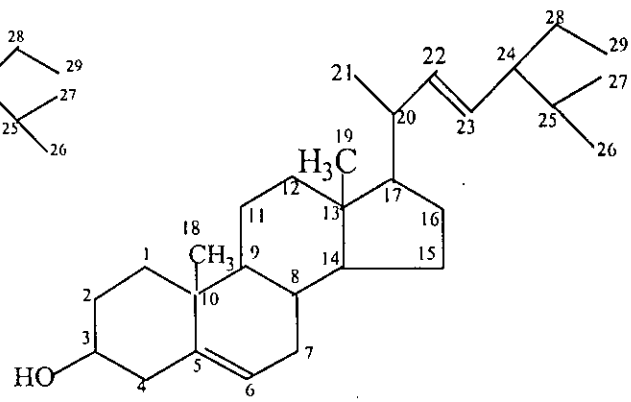
Carbon no.	Compound EA_2	Reference compound
C-1	37.24	37.30
C-2	31.65	31.70
C-3	71.80	71.80
C-4	42.31	42.40
C-5	140.73	140.80
C-6	121.70	121.70
C-7	31.90	31.90
C-8	31.87	31.90
C-9	50.15	50.20
C-10	36. 50	36.60
C-11	21. 10	21.10
C-12	39.77	39.70
C-13	42.31	42.40
C-14	56.86	56.90
C-15	24.35	24.40
C-16	29.15	29.00
C-17	56.10	56.10
C-18	12.04	12.10
C-19	19.40	19.40

C-20	40.47	40.50
C-21	21.10	21.10
C-22	138.30	138.40
C-23	129.27	129.30
C-24	51.23	51.30
C-25	31.90	31.90
C-26	21.20	21.30
C-27	19.02	19.00
C-28	25.40	25.40
C-29	12.24	12.30

Thus on the basis of all those chemical and spectral analyses, tentatively the following structure (20) may be assigned for the compound EA-2 which is quite similar with the known compound **Stigmastan-7, 22-di-ene-3β-ol** having the structure (19). Thus the compound EA-2 with the structure (20) can be named as **Stigmastan-5, 22-di-ene-3β-ol**.



Stigmastan-7,22-di-ene-3β-ol (19)



Stigmastan-5,22-di-ene-3β-ol (20)

The occurance of such a sterol molecule in plant kingdom is ubiquitous. However, the compound EA-2 with the structure (20) has been encountered for the first time in the *Tylophora indica.* In this connection it will be very much pertinent to have a brief biogenesis of the sterols. Steroids and hence sterols 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. Squalene is derived from two farnesyl pp units which must be joined in the unusual "head to head manner".

According to Fig.4.4 the polycyclic structures can be formed from squalene when squalene is folded (pseudo chair and boat conformation) on enzyme surface. This is usually initiated by acid catalyzed ring opening of squalene monoepoxide and probably occurs via a series of carbocationic intermediates. The initially formed cationic species gives stigmasterol through a series of reactions via formation of the compounds (a) and cycloartenol (b)[32].

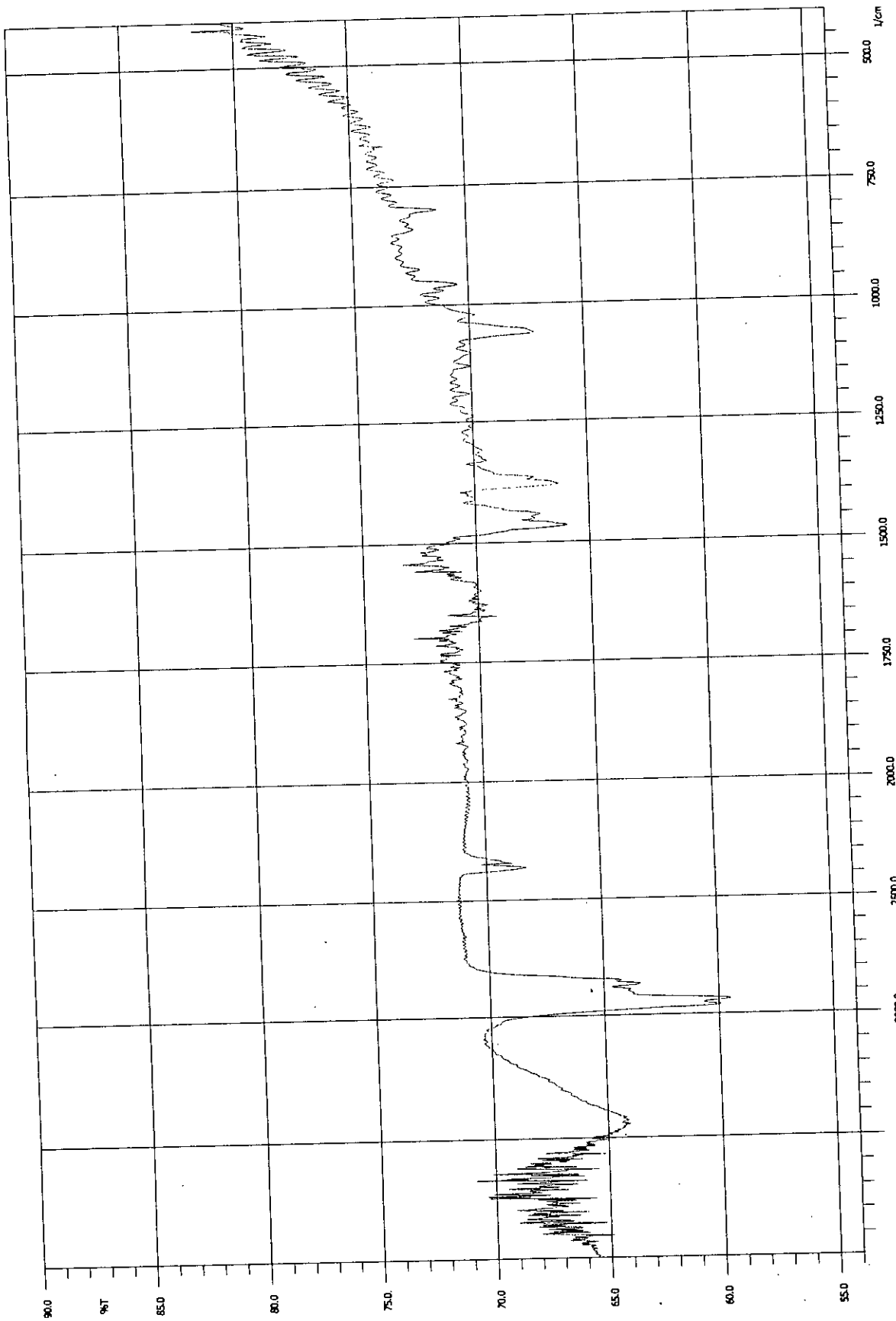


Fig 4.1: IR Spectrum of the compound EA-2:

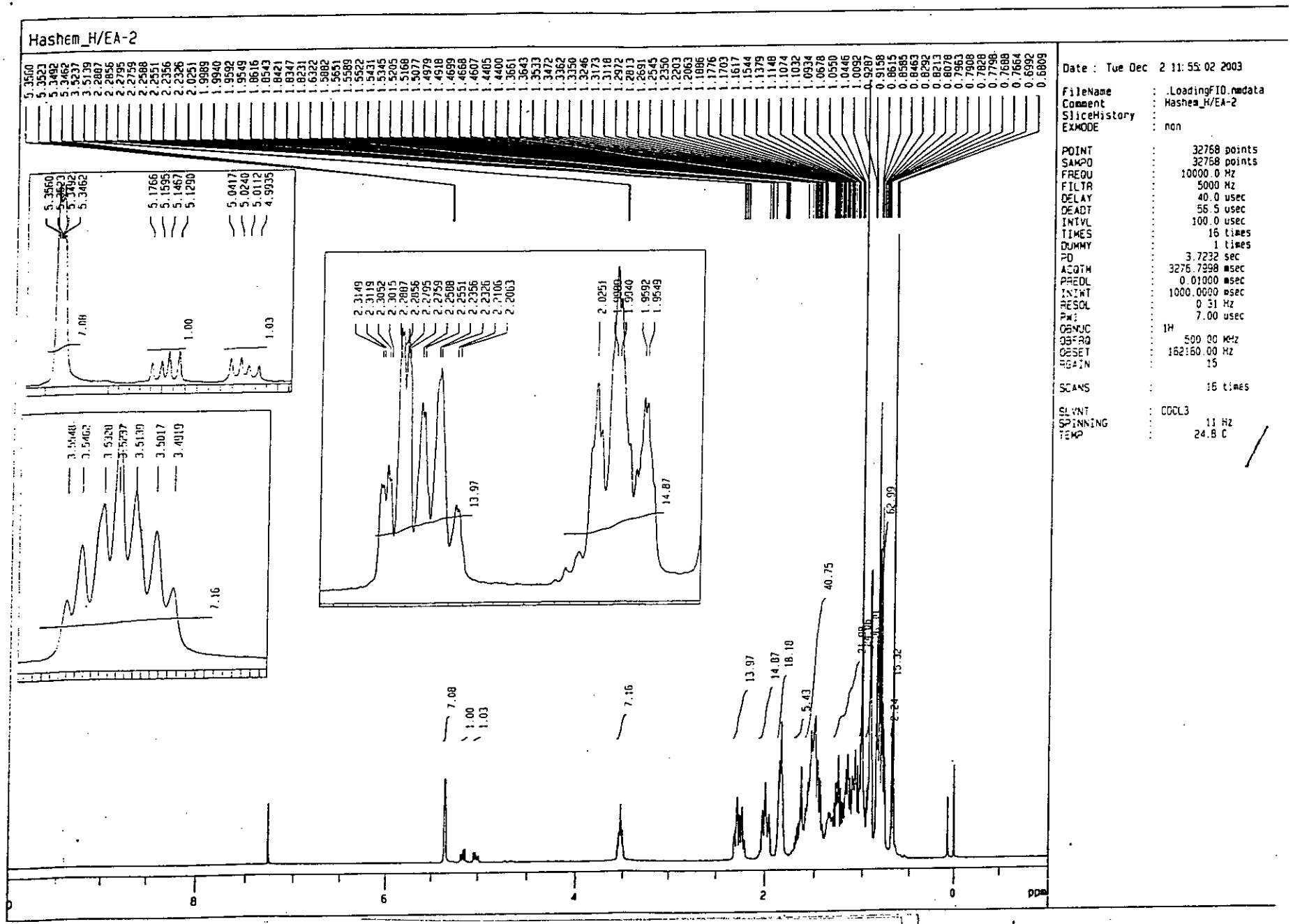


Fig.4.2a: ^{1}H-nmr spectrum of the compound EA-2:

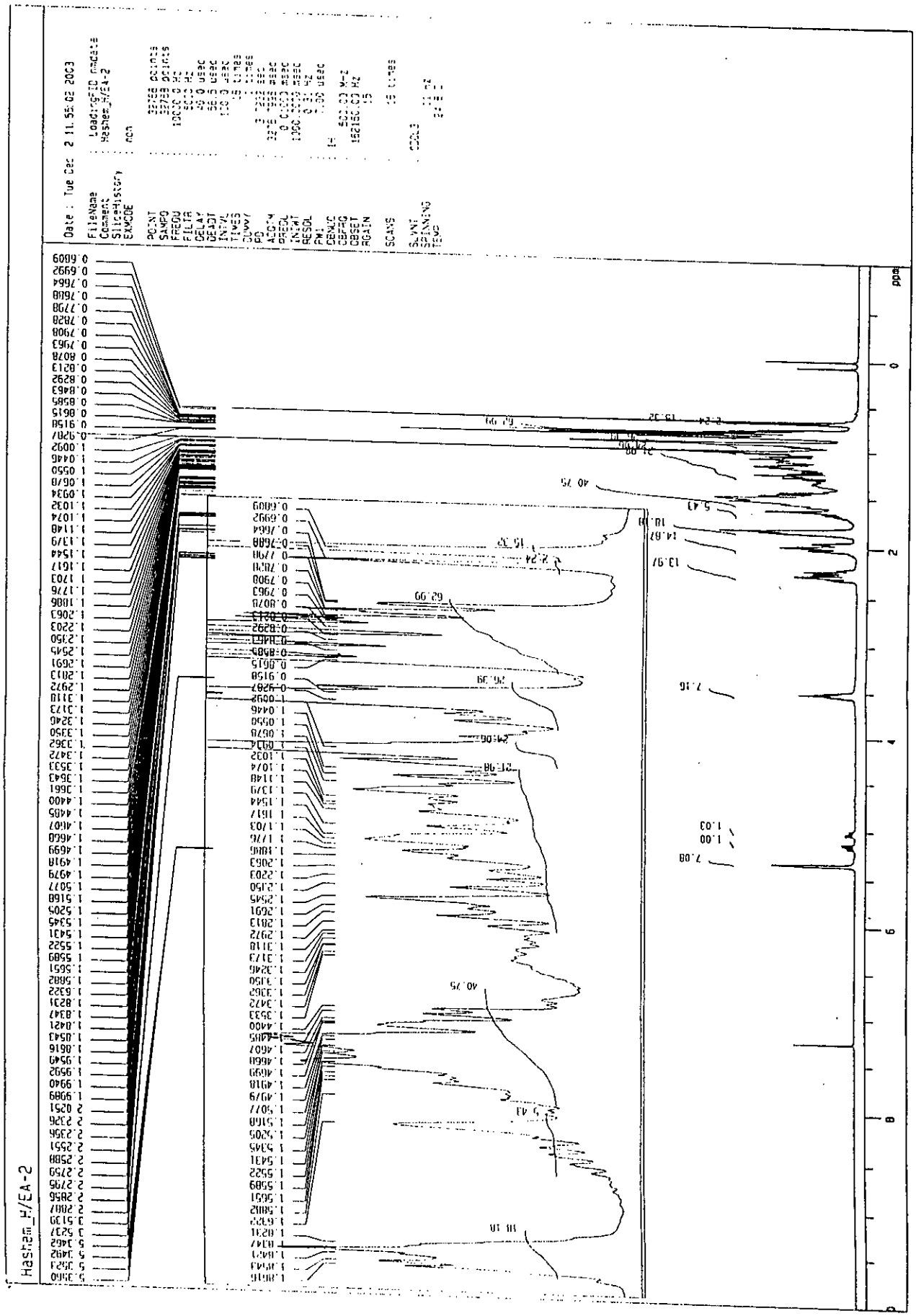


Fig.4.2b: ^{1}H- nmr spectrum of the compound EA-2:

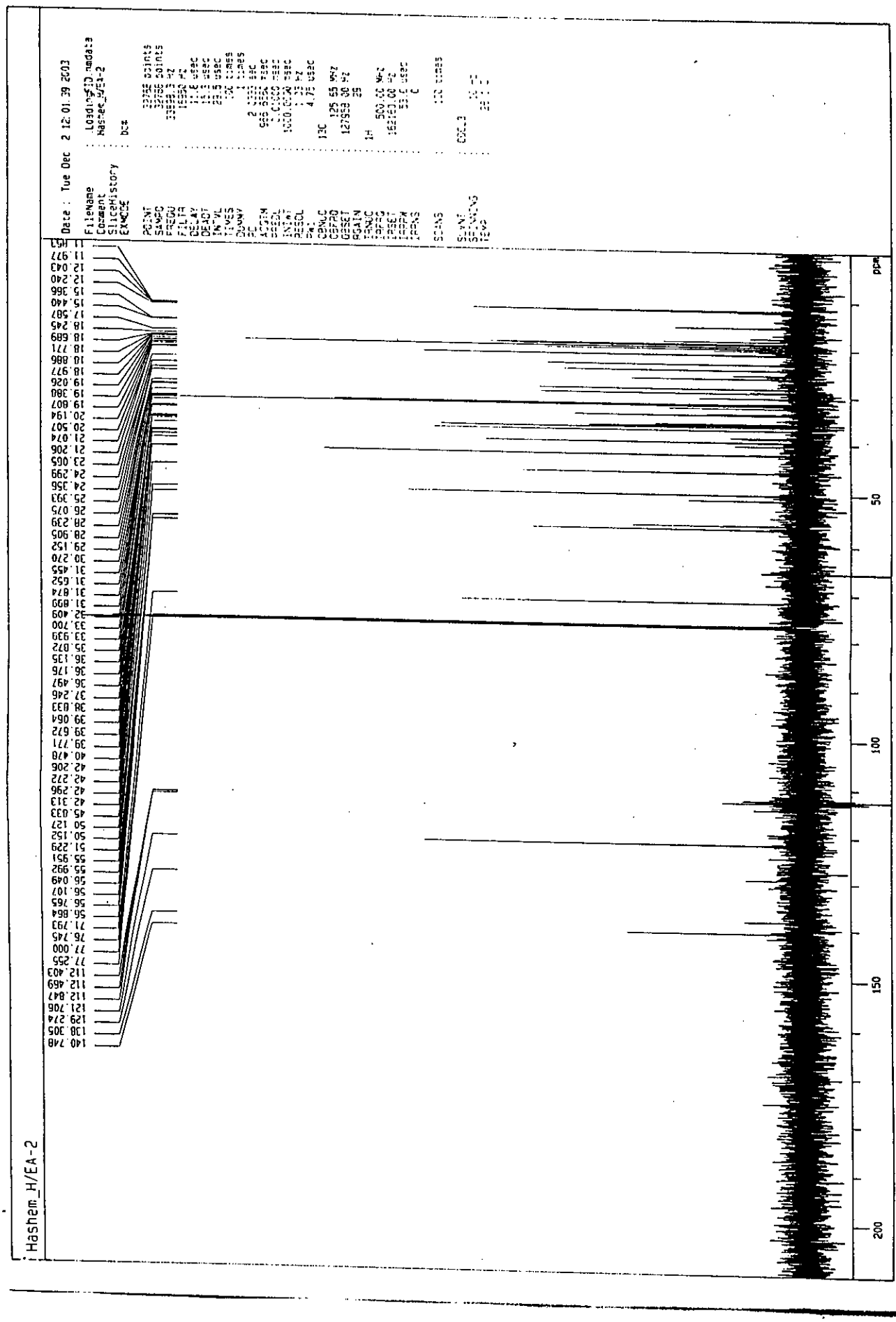


Fig.4.3a: ^{13}C- nmr spectrum of the compound EA-2:

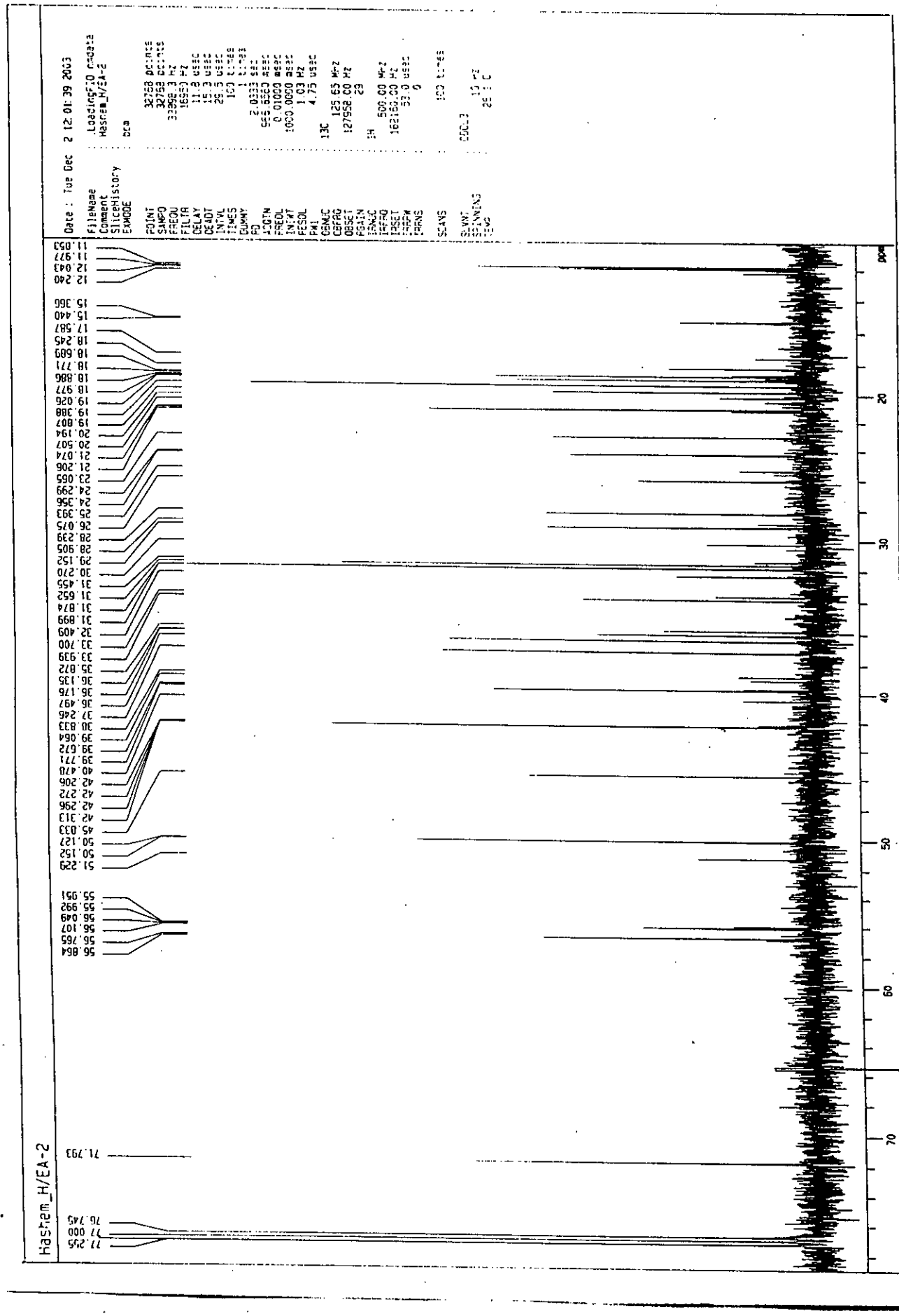


Fig.4.3b: ^{13}C- nmr spectrum of the compound EA-2:

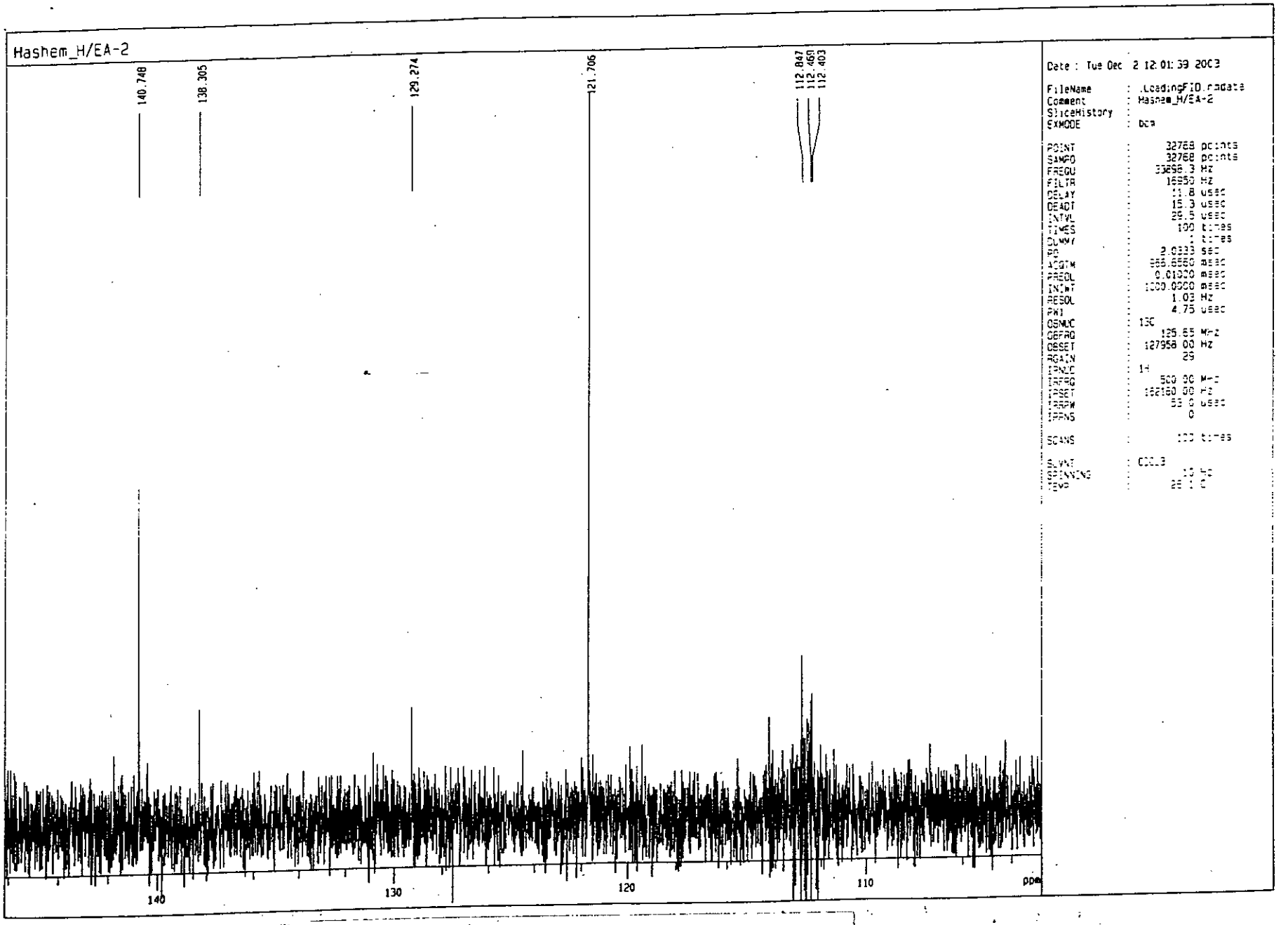


Fig.4.3c: ^{13}C- nmr spectrum of the compound EA-2:

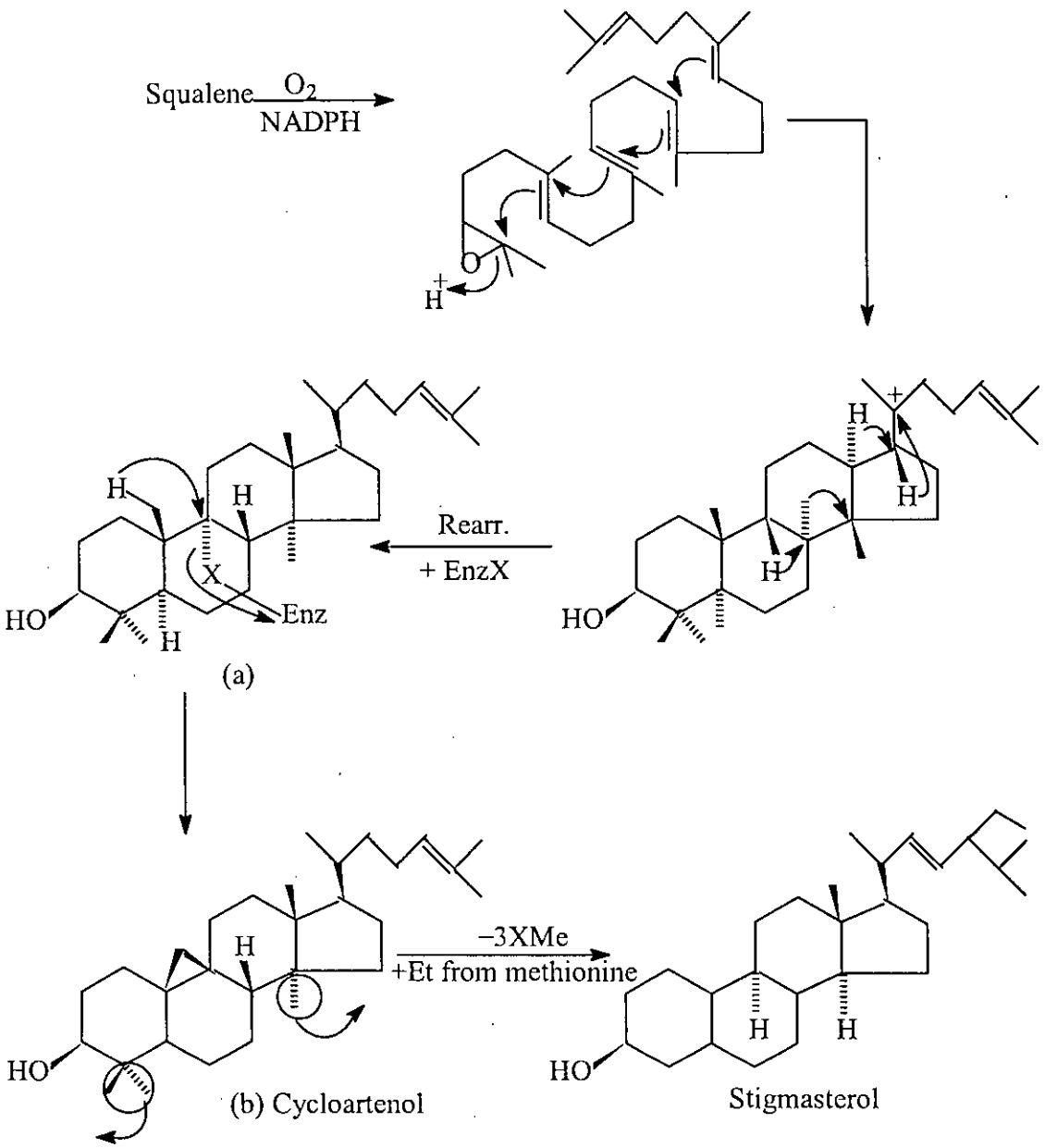


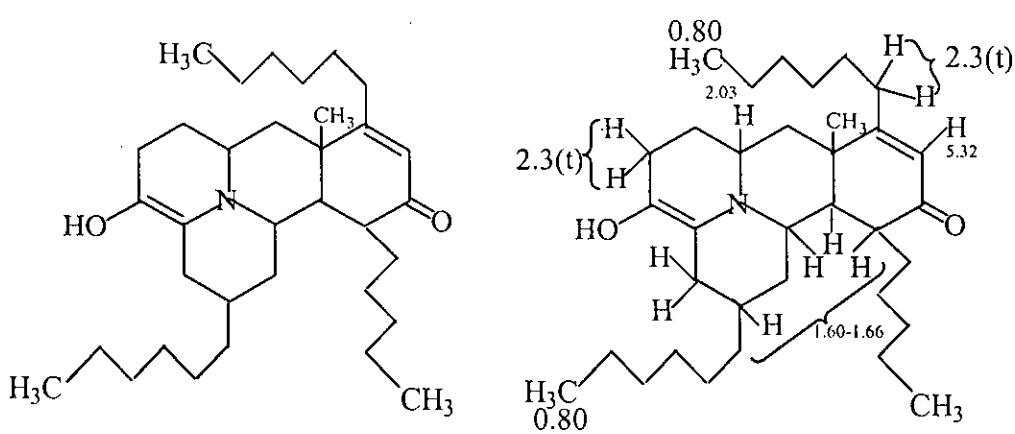
Fig. 4.4 : Possible biogenesis of stigmasterol

4.1.5 : Characterization of the compound EA-3 :

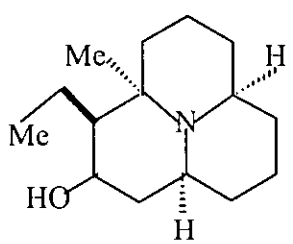
Compound EA-3 was white waxy and melts at 53-54°C. It was highly soluble in n-hexane and chloroform and also in ethyl acetate.The compound when subjected to TLC in the solvent system $CHCl_3$: EtOAc (95 : 5) gave single spot at R_f 0.62. It responded to the usual color tests for alkaloids.

The IR spectrum of the compound Fig. 4.5 showed absorption at 3500 cm^{-1} for O-H stretching. Two absorption bands at 2919 and 2849 cm^{-1} indicate C-H stretching and the band at 1450 cm^{-1} is indicative of C-H bending. The sharp absorption band at 1700 cm^{-1} is indicative of the presence of a carbonyl functional group. The absorption bands at 1300 and 920 cm^{-1} indicate stretching vibration of the C-O bond.

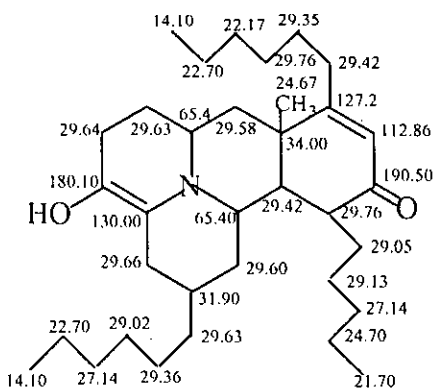
The ^{1}H-nmr spectrum Fig.4.6 showed a triplet equivalent to 6H at δ 0.88 and this is indicative of the presence of two methyl groups at the terminating points of the two chains. The multiplet at δ 5.32 for one hydrogen indicates the presence of one olefinic proton. The splitting is due to the long range coupling with $-CH_2$. The triplets at δ 2.3 for four protons are assigned for the two methylene groups attached to the two olefinic carbon atoms. The multiplet at δ 2.03 for one proton is due to the hydrogen attached to the carbon adjacent to the nitrogen atom. The four proton multiplets at δ 1.60–1.66 are assigned for the four methyne (CH) protons present in the ring system. The multiplet δ 1.18–1.45 with large intensity equivalent to 41H fits the necessary other protons required for the assigned structure of the compound EA-3.



The ^{13}C-nmr spectrum Fig. 4.7 showed the required number of carbon peaks. The peak for the carbonyl carbon is at δ 190.60. The peaks at δ 180.10 and 127.20 are for the two olefinic carbons in the rings in one of which one hydroxyl group is attached. The other two olefinic carbons are at δ 130.00 and 112.86 one of which is attached to the nitrogen atom in the ring system and the other carbon is adjacent to the carbonyl carbon. The peaks at δ 14.10, 14.10 and 21.70 are for the three methyl carbons at the terminals of the branching system. The peaks at δ 65.4 and 65.40 are for the two tertiary carbons attached to the nitrogen atom present in the ring system. The peak at δ 34.00 is for one quaternary carbon in the ring. The other peaks fit very well for the ring and chain system designed for the structure of the compound EA-3.

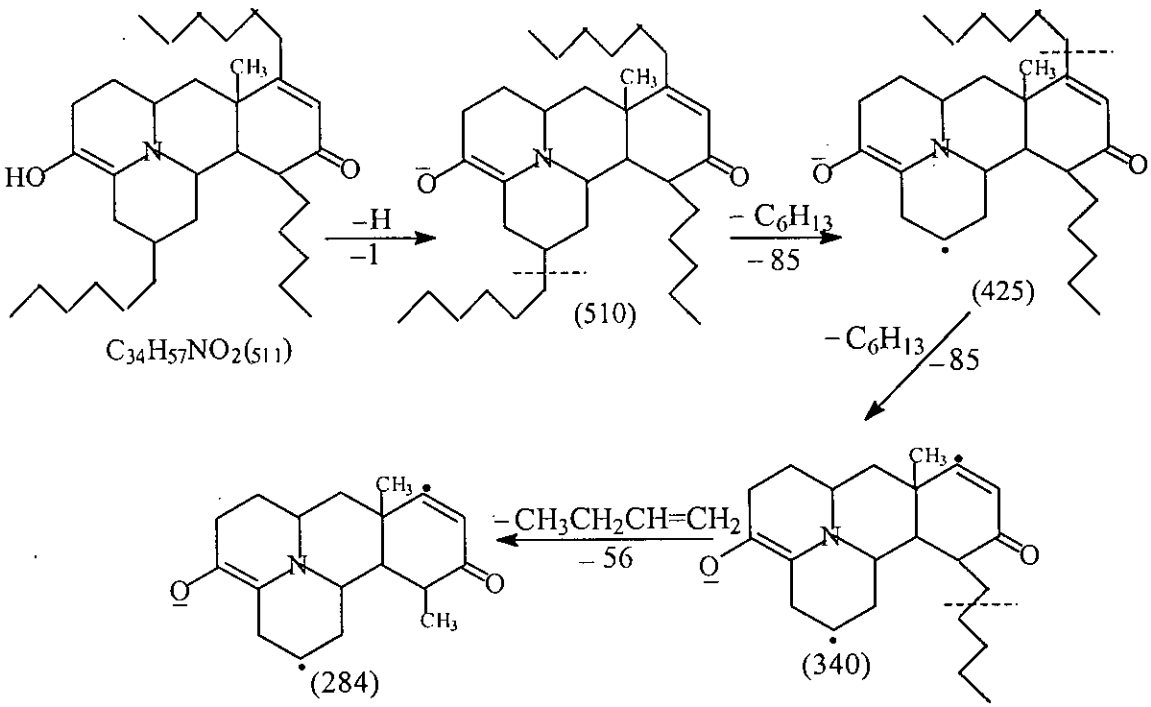


Porathericin

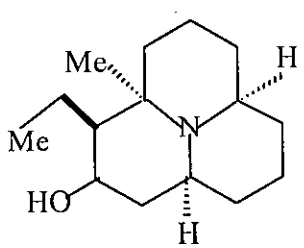


The molecular formula of the compound EA-3 calculated from the designed structure is $C_{34}H_{57}NO_2$. This requires a molecular ion peak m/z 511 but no molecular ion peak is found in the mass spectrum, Fig. 4.8. The only peak found in the mass spectrum is at m/z 284. These base can be explained on the basis of the theoretical fragmentations of $C_{34}H_{57}NO_2$ as follows .

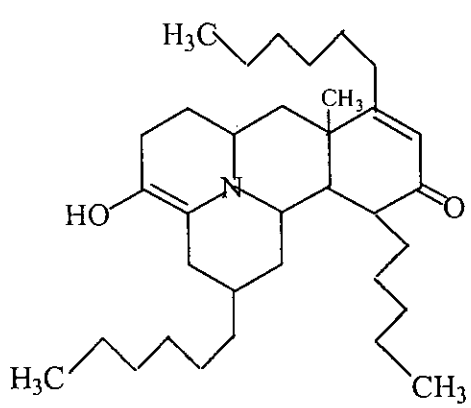
Considering molecular ion peak at m/z at 511, The m/z 510 = [M^+ - H] , The m/z 425= [M^+ - H - C_6H_{13}], the m/z 340 = [M^+ - H - C_6H_{13} - C_6H_{13}], The m/z 284 (base peak) = [M^+ - H - C_6H_{13} - C_6H_{13} - C_4H_8]



Thus with 57 protons from ^{1}H-nmr spectrum, 34 carbons from ^{13}C-nmr spectrum along with nitrogen and two oxygens, the compound EA-3 has the molecular formula $C_{34}H_{57}NO_2$ which is quite in conformity with the molecular mass 511 from which the base peak m/z 284 can be easily obtained by theretical calculations. Thus on the basis of all those chemical and spectral analyses, tentatively the following structure (22) may be assigned for the compound EA-3 and the compound EA-3 is a derivative of the alkaloid Porathericin[33] with the established structure (21)



(21)
Porathericin



(22)
Compound EA-3

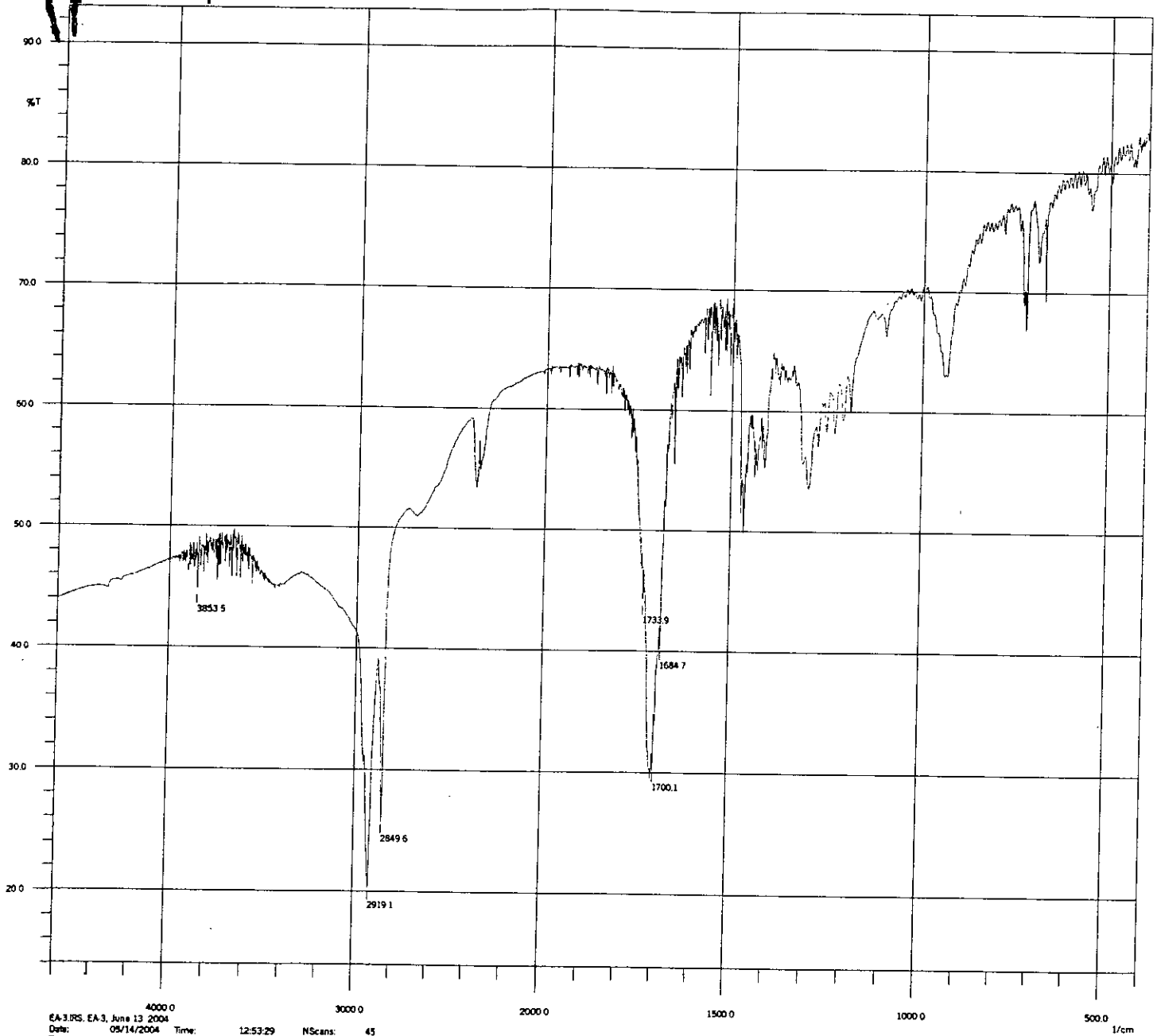


Fig 4.5: IR Spectrum of the compound EA-3:

Peaktable of EA-3.IRS, 6 Peaks
Threshhold: 45, Noise: 2, No Range Selection

No.	Pos. (1/cm)	Inten. (%T)
1	1684.7	40.195
2	1700.1	30.136
3	1733.9	43.916
4	2849.6	25.703
5	2919.1	20.245
6	3853.5	44.462

EA-3, June 13. 2004

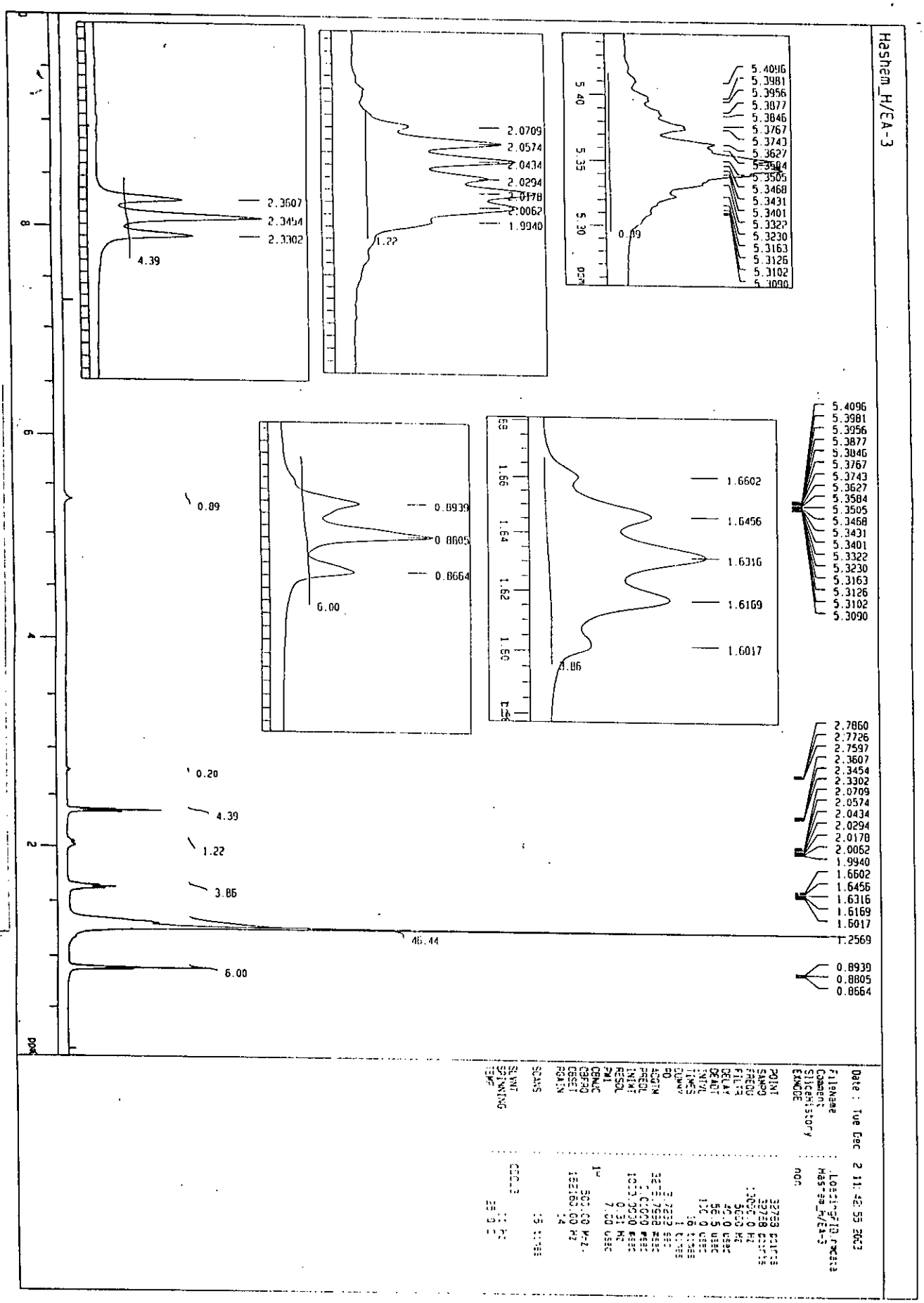


Fig.4.6: ^{1}H- nmr spectrum of the compound EA-3:

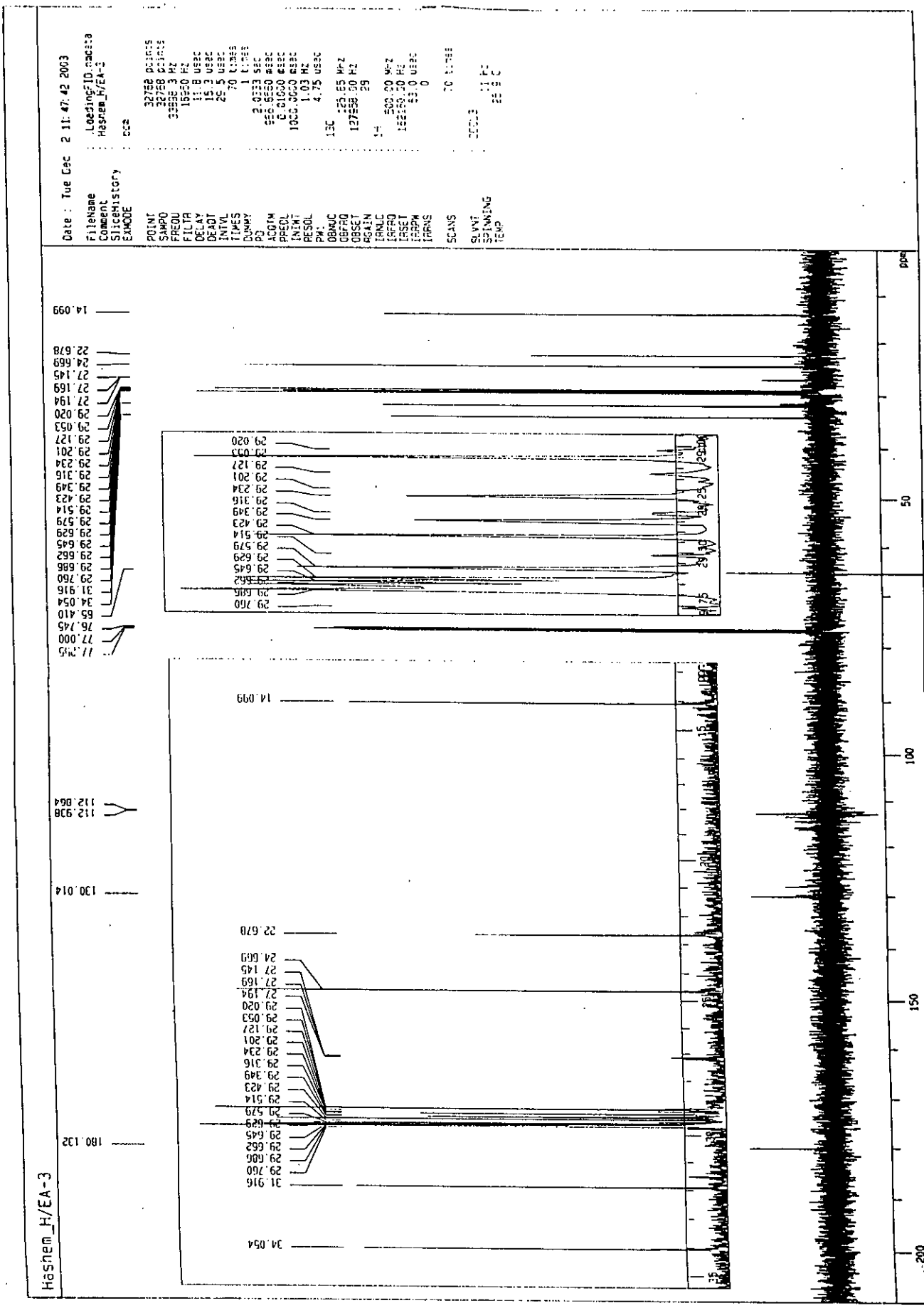


Fig.4.7: ^{13}C- nmr spectrum of the compound EA-3:

#:1　保持時間:9.783(スキャン#:695)
ピーク数:2　ベースピーク:281.80(1368)
スペクトル:シングル 9.783(695)
バックグラウンド:なし

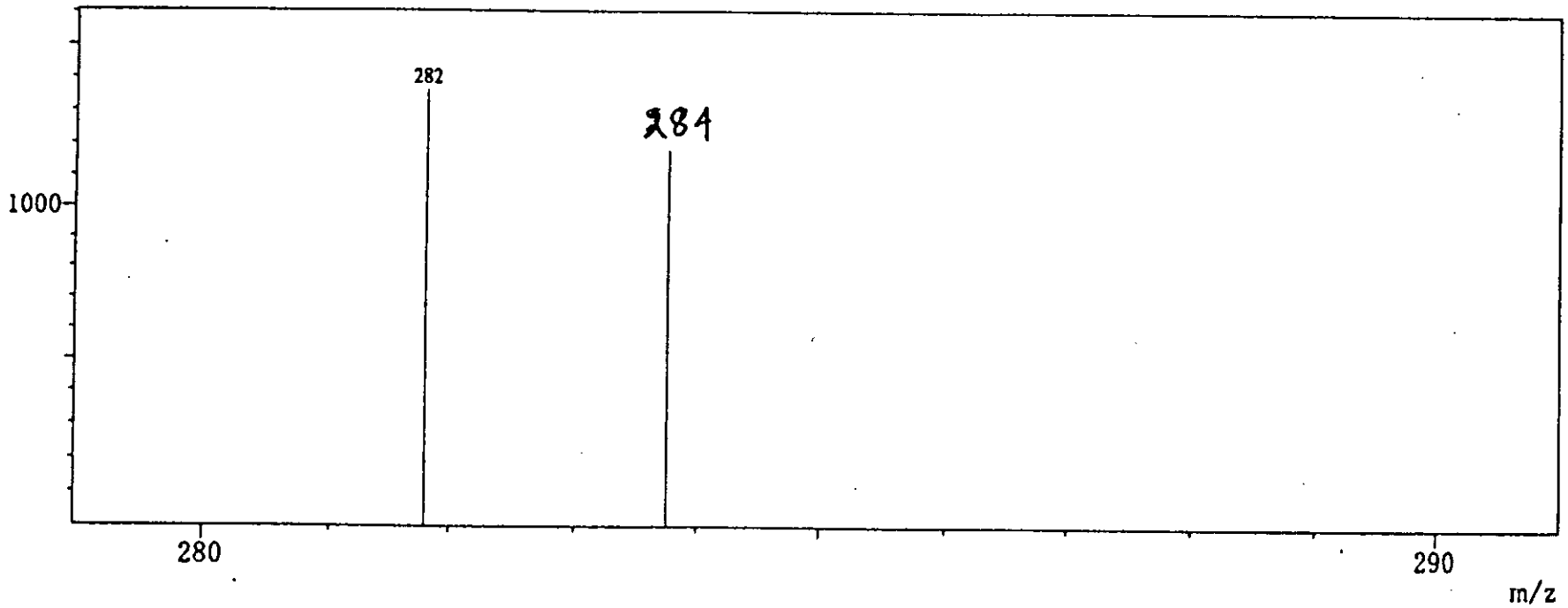


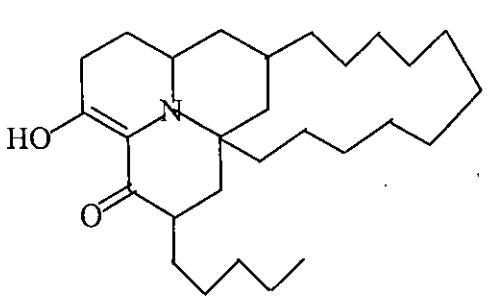
Fig 4.8: Mass spectrum of the compound EA-3:

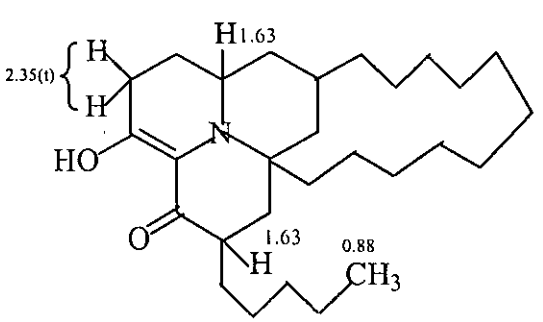
4.1. 6 : Characterization of the compound EA-4 :

The compound EA-4 was a white powder. It melts at 70-71°C. The compound is soluble in n-hexane, chloroform and also in ethyl acetate. Its TLC in the solvent system n-hexane : EtOAc (80 : 20) showed a single spot with a R_f value 0.5. It responded to the usual color tests for alkaloids.

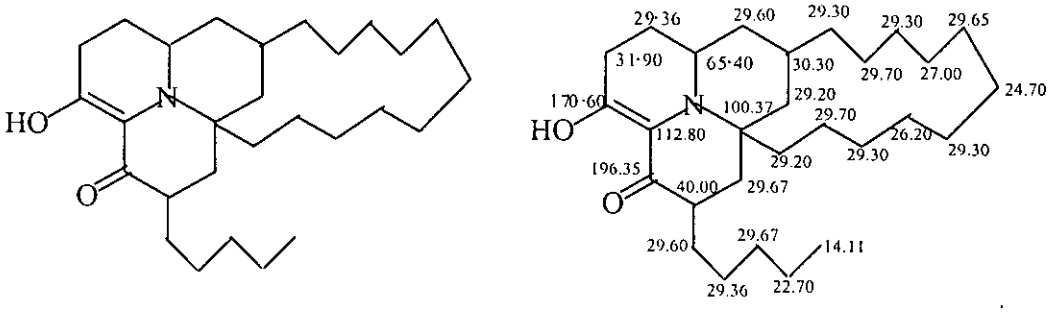
The IR spectrum of the compound EA-4 Fig. 4.9 showed one broad absorption band at 3300 cm^{-1} for the OH group. The bands at 2917 and 2849 cm^{-1} are for C-H stretching vibration. A sharp absorption band at 1700 cm^{-1} is indicative of the carbonyl group present in the compound. The band at 1630 cm^{-1} is for the olefinic double bond. The bands at 1460 and 1445 cm^{-1} are for C-H bending and the band at 1280 cm^{-1} is for C-N stretching.

The ^{1}H-nmr spectrum Fig.4.10 showed a triplet at δ value 0.88 for three protons of one methyl group present at the terminal of the side chain. Another triplet at δ value 2.35 is equivalent to two protons of methylene group attached to the olefinic carbon. A multiplet at δ 1.63 is equivalent for two 2H proton of methyne groups one of which is attached to nitrogen atom and the other proton is attached to the carbon adjacent to the carbonyl group in the ring system. Other broad multiplets at δ 1.18-1.45 are for 40 protons equivalent to nineteen methylene groups, one methyne group and one hydroxyl group present in the compound.

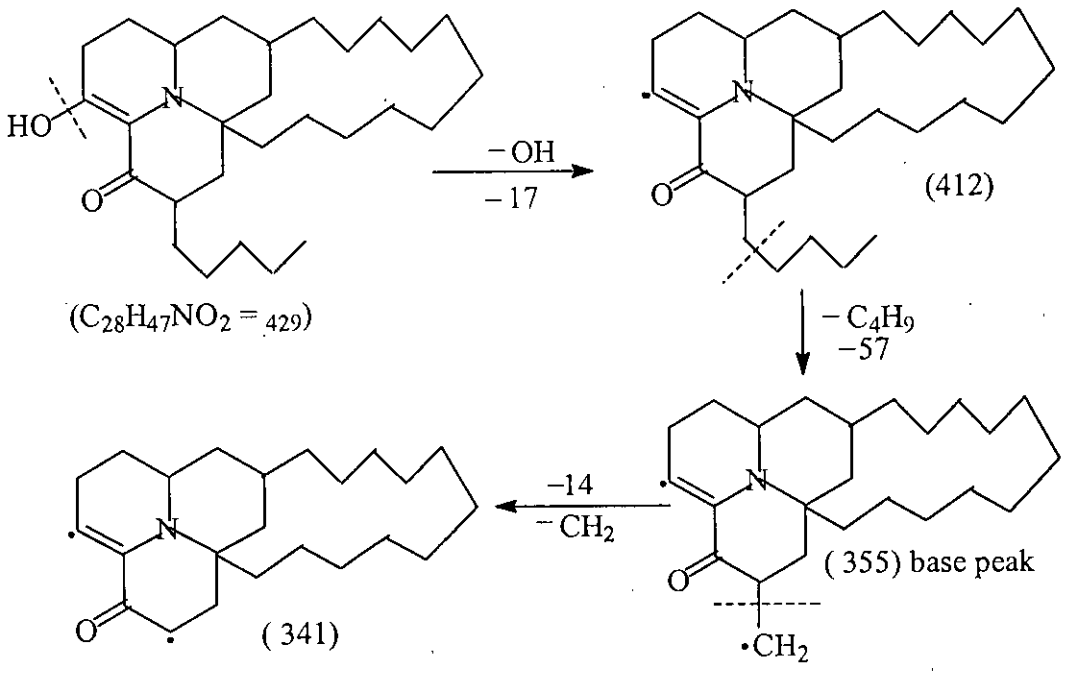




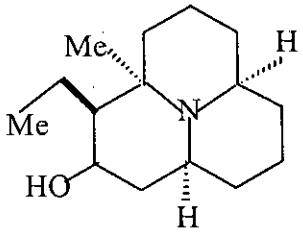
^{13}C-nmr spectrum of the compound EA-4 Fig. 4.11 showed one peak δ 196.35 for the carbonyl carbon. The olefinic carbons are at δ 170.6 and 112.80. The peak at δ 100.37 ppm.is for the quaternary carbon in the ring. The peak at δ 65.40 is for the tertiary carbon adjacent to the nitrogen atom in the ring. The other peaks at δ 14.11, 22.70, 24.70, 26.20, 27.00, 29.20, 29.20, 29.30, 29.30, 29.30,29.30, 29.36, 29.60, 29.60, 29.65, 29.67, 29.67, 29.70, 29.70, 30.30, 31.90 and 40.00 are for methyne, methylene and methyl carbons present in the ring and side chain.



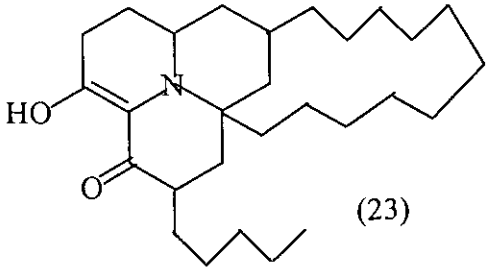
The molecular formula of the compound EA-4 is $C_{28}H_{47}NO_2$.The mass spectrum of EA-4 Fig.4.12 shows the molecular ion peak at m/z 429 which exactly fits its molecular formula and structure. The base peak at m/z 355 can be easily explained by the fragmentation of hydroxyl and n-butyl group from the side chain. The fragmentation of another methylene group from the base peak gave the peak at m/z 341. The other peaks of the mass spectrum can also be explained on considering the assigned structure EA-4. The fragmentations can be schematically shown as follows :



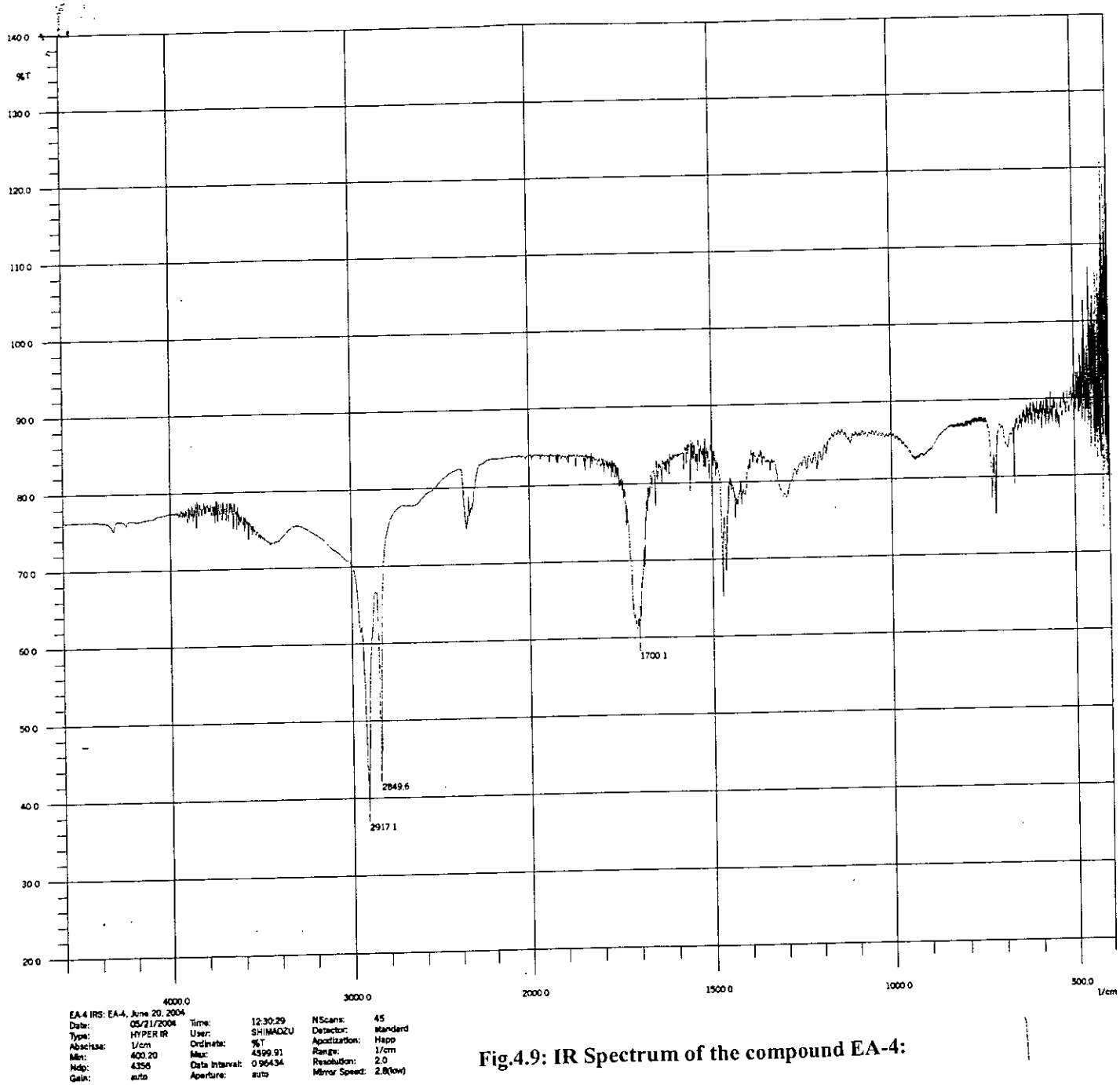
From the analysis of the IR spectrum, counting 47 protons from ^{1}H-nmr spectrum and 28 carbons from ^{13}C-nmr spectrum along with one nitrogen and two oxygens, the molecular formula of the compound EA-4 can be written as $C_{28}H_{47}NO_2$ .The calculated molecular weight 429 of the compound is in agreement with the molecular ion pick at m/z 429 in the mass spectrum.Considerinng all these things, the following structure (23) may be tentatively assigned for the compound EA-4.This compound EA-4 is also a **porathericin (21)** derivative[33] like our isolated compound EA-3.



(21)
Porathericin



(23)
Compound EA-4



Peaktable of EA-4.IRS, 3 Peaks
Threshhold: 65, Noise: 2, No Range Selection

No.	Pos. (1/cm)	Inten. (%T)
1	1700.1	59.609
2	2849.6	43.460
3	2917.1	38.308

EA-4, June 20. 2004

Fig.4.9: IR Spectrum of the compound EA-4:

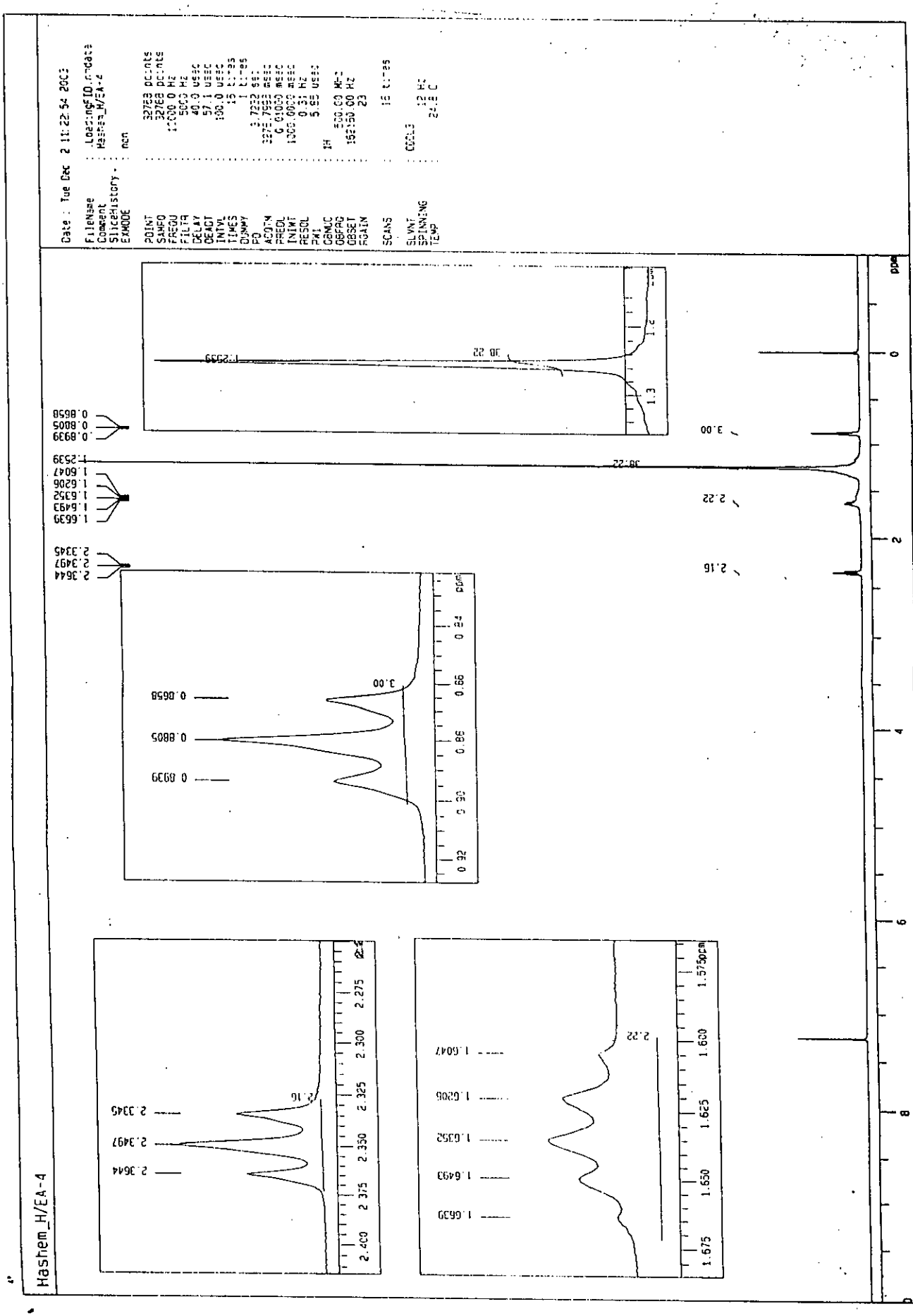


Fig.4.10: ^{1}H- nmr spectrum of the compound EA-4:

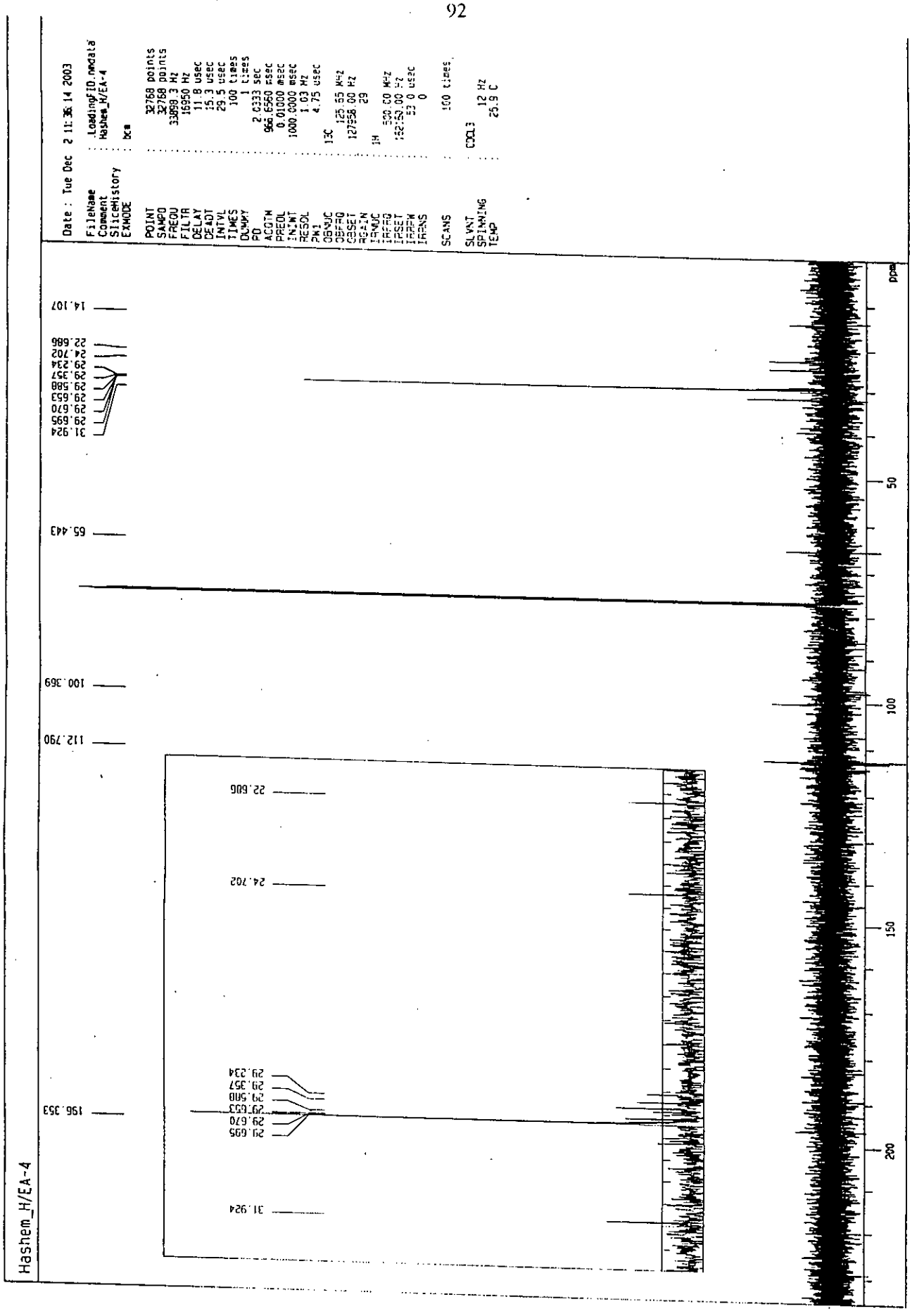


Fig.4.11: ^{13}C- nmr spectrum of the compound EA-4:

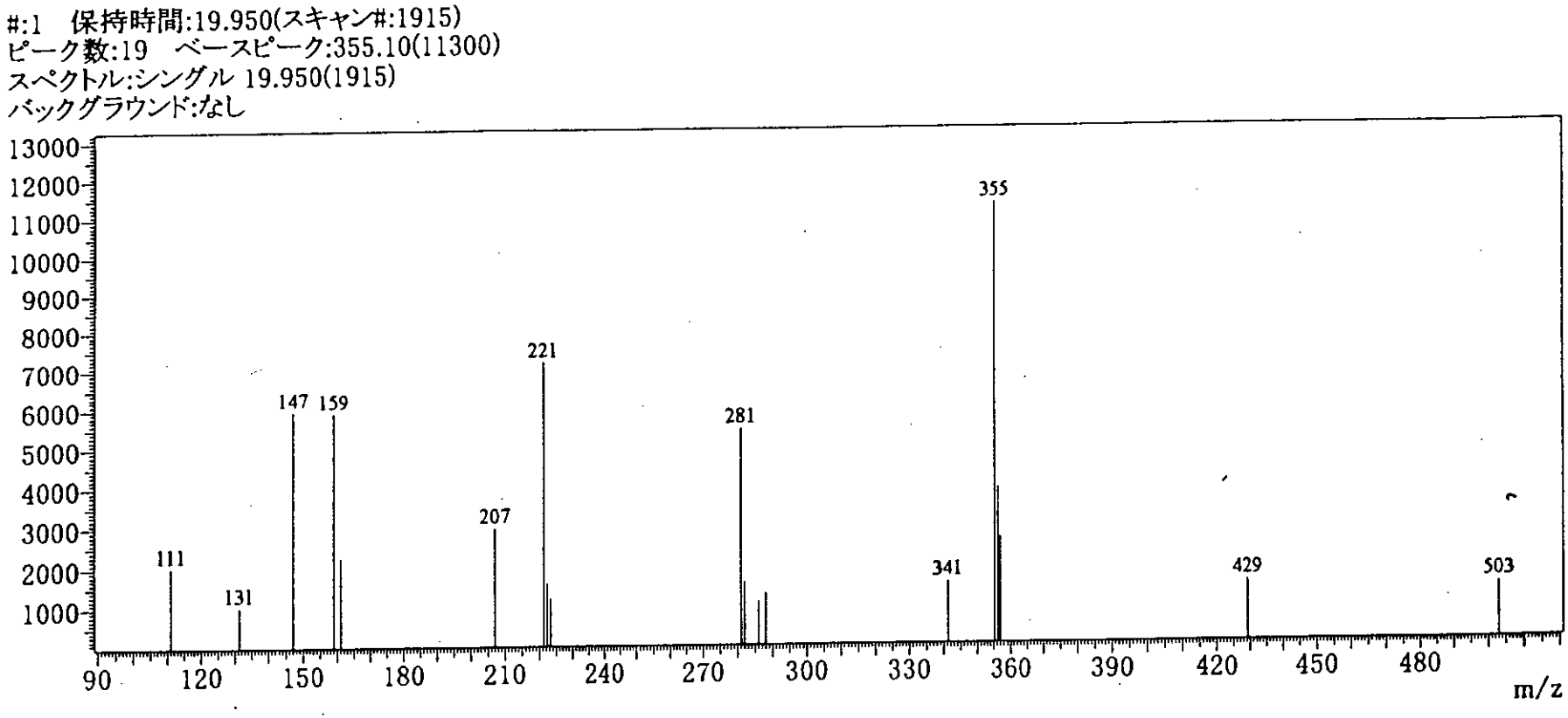


Fig 4.12: Mass spectrum of the compound EA-4:

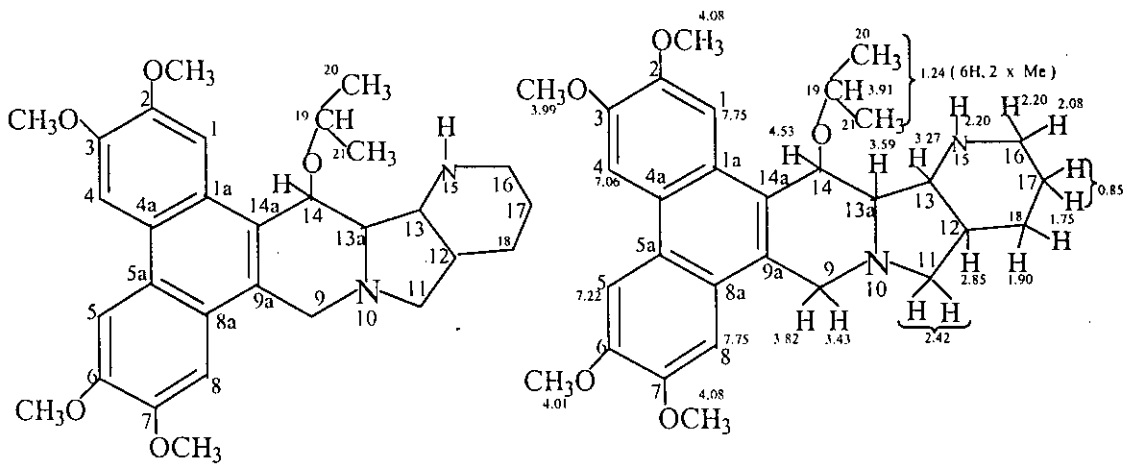
4.1.7 : Characterization of the compound A_5 :

The compound A_5 was a deep orange powder. It melts at 178-180°C. It was soluble in chloroform, ethyl acetate and methanol. The TLC of the compound in the solvent MeOH : EtOAc : $CHCl_3$ (50 : 40 :10) gave single spot at R_f value 0.65. It responded to the usual color tests for alkaloids.

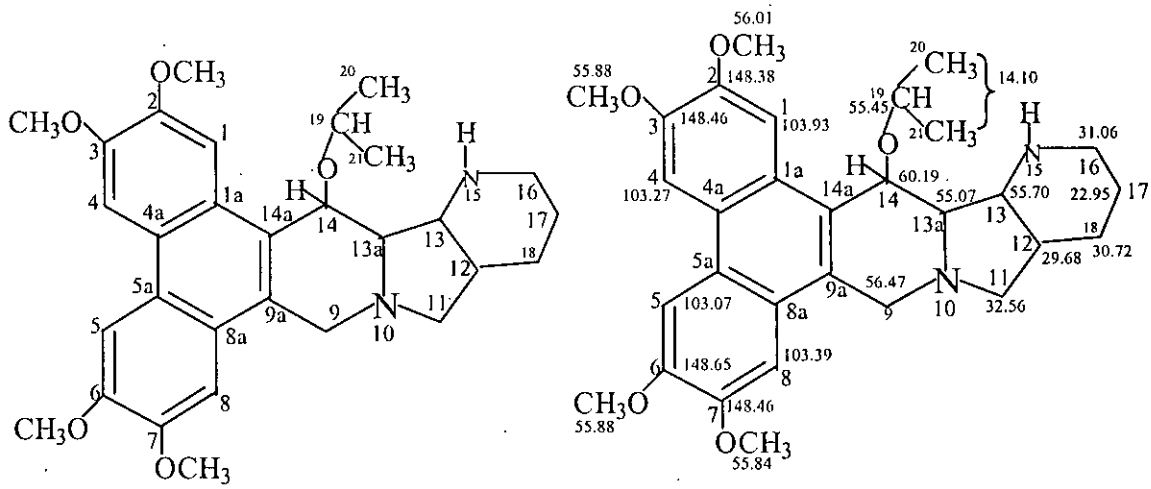
The IR spectrum Fig. 4.13 of the compound A_5 showed a broad absorption at 3300cm^{-1} for N-H stretching. It also showed an absorption at 3010 cm^{-1} for olefinic =C-H stretching. The absorptions at 2920 and 2840 cm^{-1} are for alkane –C-H stretching and the 1467, 1442, 1425 cm^{-1} due to the C-H bending vibrations. The absorption bands at 1616 and 1512 cm^{-1} indicate the presence of aromatic double (C=C) bond stretching. The sharp absorption bands at 1147 and 1195 cm^{-1} are due to C-N stretching vibration. The bands at 1035 and 1016 cm^{-1} are indicative of the C-O stretching vibration.

The ^{1}H-nmr spectrum Fig. 4.14 (a,b,c,d) showed one singlet at δ 7.75 for two aromatic protons and another two singlets at δ 7.22 and 7.06 are for other two aromatic protons. The methoxy groups attached to the aromatic ring showed a sharp singlet equivalent to 6H at δ 4.08 and another two singlets each of 3H at δ 4.01 and 3.99. The doublet at δ 4.53 is for the 14-H and the doublet at δ 3.82 is for 9-H. Another 9-H is at δ 3.43 as doublet. The doublet of doublet at δ 3.59 is for the 13a-H. The doublet of doublet at δ 3.27 is indicative of 1H of 13-H and another doublet of doublet at δ 2.82 is indicative of 1H for 12-H.The 11,11-H equivalent to 2H appeared as multiplet at δ 2.42. The multiplet at δ 2.20 (1H) was for the 16-H. Another broad absorption at δ 2.20 (1H) is assigned for N-H. The multiplet at δ 2.08 (1H) indicates the presence of proton at C-16. The septet at δ 3.91 (1H) indicates the presence of isopropyl group at (C-19) which is attached with C-14 through an oxygen atom making an ether linkage. The multiplet at δ 1.90 (1H) was due to one of the methylene protons at C-18 atom. The other methylene proton of C-18 is at δ 1.75. The sharp singlet at δ 1.24 (6H) was for two methyl groups at C-20,21.

Another multiplet at δ 0.85 (2H) indicates the presence of methylene protons of carbon C-17. Thus the proton signals clearly fit the structure given below for the compound A_5.



The ^{13}C-nmr spectra Fig. 4.15 (a,b,c,d) showed peaks at δ148.65, 148.46 and 148.38 for [C-2,3,6,7) in the aromatic ring system. The values at δ 126.18, 125.73, 124.23, 123.57, 123.38 are for the carbon atoms [C-1a, 4a, 5a, 8a, 9a, 14a] and the signals at δ 103.93, 103.39,103.27, 103.07 are for the carbon atoms [C-1,4,5,8] in the ring system. The signals as –CH and tertiary carbons are clearly identified by comparing ^{13}C-nmr and ^{13}C-Dept spectra. The signals at δ 60.19 is for the carbon attached to the ring containing the isopropoxy group. The signals at δ 56.01, 55.88, 55.84 are for the four methoxy groups present in the aromatic ring system. The other signals at δ 56.47 (C-9), 55.70 (C-13), 55.45 (C-19), 55.07 (C-13a), 32.56 (C-11), 31.06 (C-16), 30.72 (C-18), 29.68 (C-12), 22.95 (C-17) and 14.10 (C-20, 21) fit very well the proposed carbon skeleton of the compound A_5.



The carbon signals of the compound A_5 were assigned on the basis of the ^{13}C-Dept signals Fig. 4.15 (a, b). When the dept spectra of the compound A_5 was compared with those of the normal ^{13}C-nmr, it is found that there are 10 tertiary carbons, 6 methyls (4 x OCH_3, 2 x CH_3), 5 methylene (2 x N-CH2, 3 x CH2) and 9 methyne (4 x CH aro., 2 x OCH, 2 x N-CH, 1 x CH) carbons which exactly fit the proposed structure of the compound A_5.

The arrangement of protons in the compound A_5 was also confirmed from the ^1H-^1H COSY spectra Fig 4.16 (a,b,c Table-4.2). The spectra exhibited cross peaks between δ 4.53 (H-14) ↔3.59(H-13a) and δ 3.82 (H_a-9) ↔3.43(H_b-9) which confirmed the protons of ring D. The cross peaks between δ 3.91 (H-19) ↔1.24(H-20,21) confirmed one isopropyloxy group attached to the ring D. The spectra also exhibited cross peaks between δ 2.42(H-11) ↔2.85(H-12), 3.43(H_b-9); 2.85(H-12) ↔3.27(H-13),2.42(H-11);and 3.27(H-13) ↔ 2.85(H-12), 3.59(H-13a), 2.42(H-11) confirmed the protons of ring E. The cross peaks between δ 1.90 (H-18) ↔ 2.42 (H-11), 2.08 (H-16) ; δ 2.20 (H_a-16) ↔ δ 2.08 (H_b-16), 2.42 (H-11), 1.90 (H_a-18) and δ 0.85 (H –17) ↔ δ 1.90 (H-18), 2.20 (H-16) confirmed the presence of 3 methylene groups in the ring F.

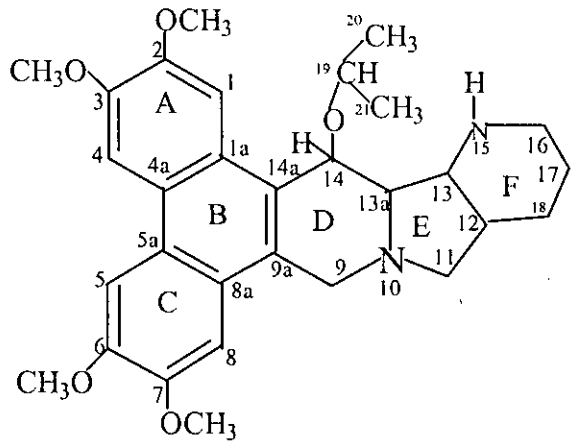


Table 4.2 : ^{1}H-^{1}H Cosy spectral data of A_5 :

Signals for ^{1}H-nmr	↔	Signals for ^{1}H-nmr
4.53 (H-14)	↔	3.59 (H-13a)
3.91 (H-19)	↔	1.24 (H-methyl, 20,21)
3.59 (H-13a)	↔	3.27 (H-13)
3.27 (H-13)	↔	3.59(H-13a), 2.85(H-12), 2.42 (H-11)
3.82 (Ha–9)	↔	3.43 (H_b-9)
2.85 (H-12)	↔	3.27(H-13), 2.42(H-11)
2.42 (H-11a)	↔	2.85 (H-12), 3.43 (H_b-9)
2.20 (H_a-16)	↔	2.08 (H_b-16), 2.42 (H-11), 1.90 (H_a-18)
1.90 (H_a-18)	↔	2.08 (H_b-16), 2.42 (H-11)
0.85 (H-17)	↔	1.90(H_a-18), 2.20(H_a-16)

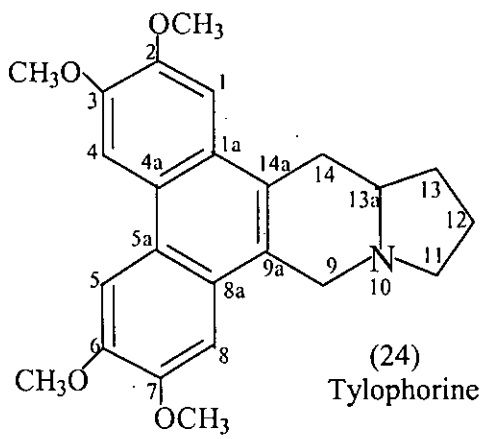
The HMBC spectra Fig 4.18 (Table 4.3) of the compound showed the cross peaks at δ 7.75 (H-1,8) ↔ δ 103.93 (C-1), 103.39 (C-8) ; δ 7.22 (H-5) ↔ δ103.07 (C-5) and δ 7.06 (H-4) ↔ δ103.27 (C-4) indicate the protons and carbons at the positions 1,4 of the ring A and 5,8 of the ring C. The cross peaks at δ 4.08, 4.01, 3.99 (4 x OCH_3 at C-2,3,6,7) ↔ δ 57.37, 57.25, 57.21, 57.09 (4 x OCH_3) , δ 148.65, 148.46, 148.38 (C-

2,3,6,7) indicate the protons and carbons at the positions 2,3 of the ring A and 6,7 of the ring C and this also confirms the positions of the four methoxy groups in the two rings A and C. The cross peaks at δ 4.53 (H-14) ↔ δ 60.19 (C-14) ; δ 3.91(H-19) ↔ δ 55.45 (C-19); δ 3.59 (H-13a) ↔ δ 55.07 (C-13a) ; δ 3.27 (H –13) ↔ δ 55.70(C-13) ; δ 2.85 (H-12) ↔ δ 29.68 (C-12) together with the other cross peaks given in the table 4.2 clearly confirms the rest of the skeleton structure of the compound A_5.

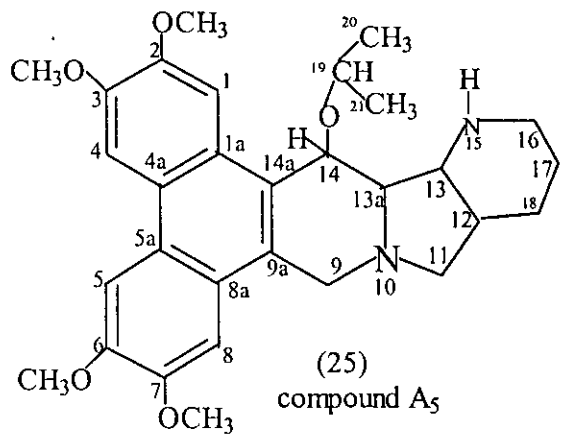
Table 4.3 : ^{1}H-^{13}C Hetero-Cosy (HMBC) spectral data of A_5 :

Signals for ^{1}H-nmr	↔	**Signals for ^{13}C-nmr**
7.75 (H-1, 8)	↔	103.93 (C-1), 103.39 (C-8)
7.22 (H-5)	↔	103.07 (C-5),
7.06 (H- 4)	↔	103.27 (C-4)
4.53 (H-14)	↔	60.19 (C-14), 148.38 (C-2)
4.08,4.01,3.99	↔	57.37, 57.25, 57.21, 57.09 (4 x OCH_3)
4.08,4.01,3.99 (4 x OCH_3 at C-2,3,6,7)	↔	57.37, 57.25, 57.21, 57.09 (4 x OCH_3) 148.65, 148.46, 148.38 (C-2,3,6,7)
3.91(H-19)	↔	55.45 (C-19)
3.82(H_a-9)	↔	56.47 (C-9)
3.59 (H_a-13a)	↔	55.07 (C-13a)
3.43 (H_b-9)	↔	56. 47 (C-9)
3.27 (H_b-13)	↔	55.70 (C-13)
2.85 (H-12)	↔	29.68 (C-12)
2.42 (H-11)	↔	32.56 (C-11)
1.24 (H-20,21)	↔	14.10 (C-20,21)
0.85 (H-17)	↔	22.95 (C-17)

Thus with 38 protons from ^{1}H-nmr spectrum, 30 carbons from ^{13}C-nmr and ^{13}C - Dept spectra along with the functional groups from IR spectrum, the molecular formula of the compound A_5 can be written as $C_{30}H_{38}N_2O_5$ .Though the mass spectra of the compound is not available, considering its available ^{1}H-^{1}H COSY and ^{1}H -^{13}C COSY(HMBC), the following structure (25) may be tentatively assigned for the compound A_5 with the molecular formula $C_{30}H_{38}N_2O_5$. This compound A5 with the designed structure (25) is a derivative of the alkaloid **Tylophorine**[24] with the established structure(24).



(24)
Tylophorine



(25)
compound A_5

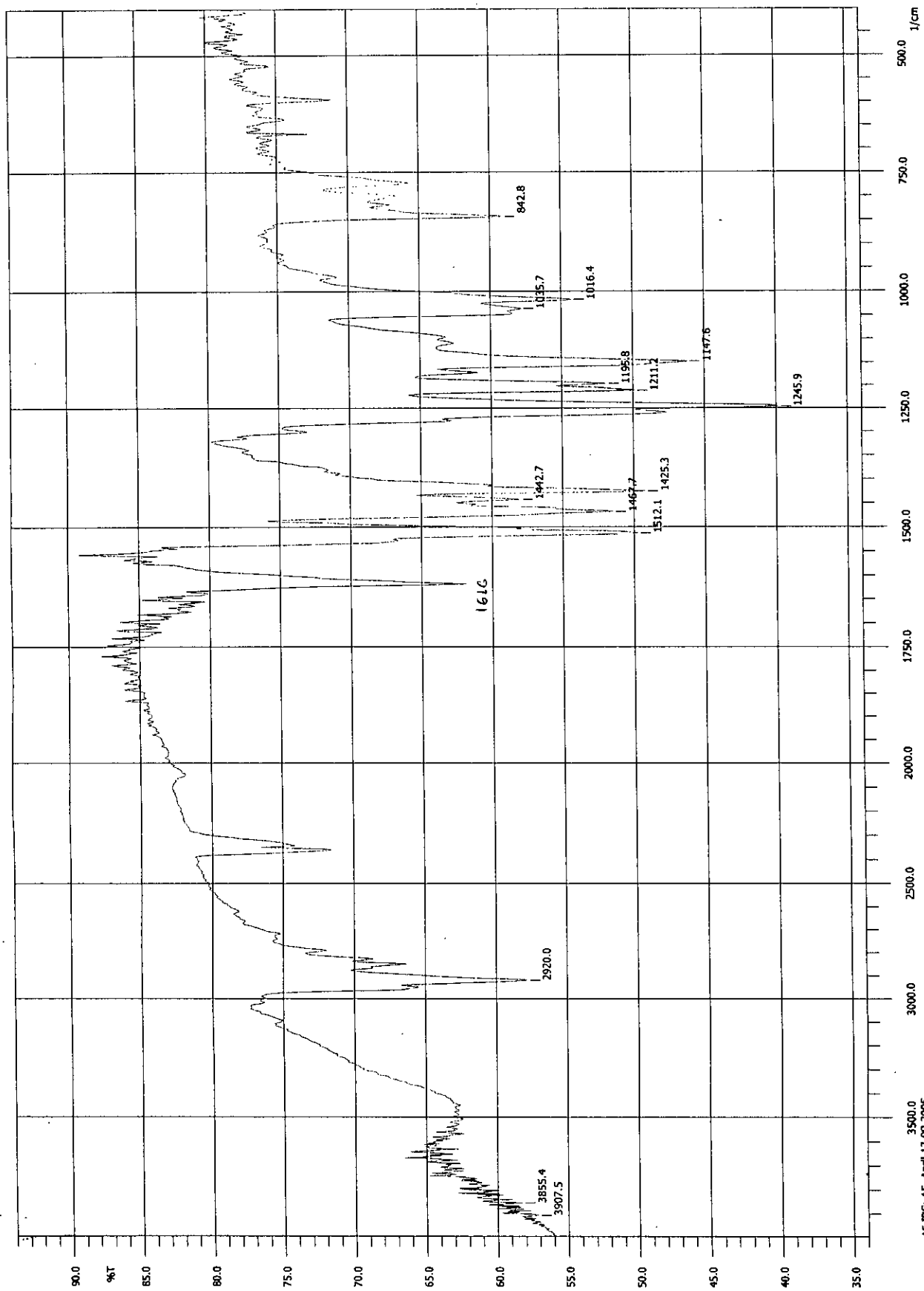


Fig 4.13: IR Spectrum of the compound A_5

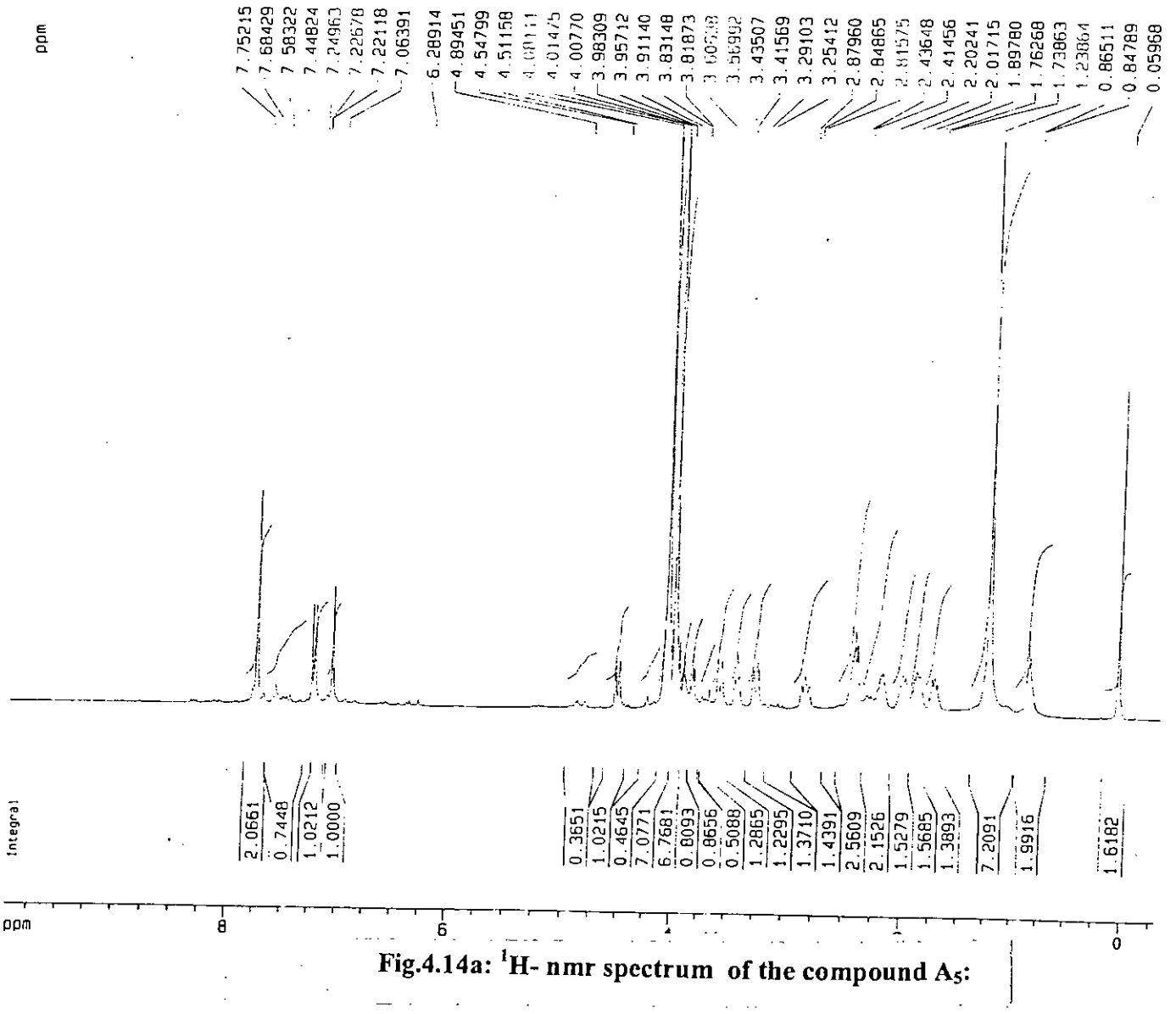


Fig.4.14a: ^{1}H- nmr spectrum of the compound A_5:

```
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NAME                  A1789
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_              20050428
Time                  11.31
INSTRUM              dpx400
PROBHD     5 mm Multinuc
PULPROG               zg30
TD                   32768
SOLVENT              CDCl3
NS                     128
DS                       2
SWH              4930.966 Hz
FIDRES           0.150461 Hz
AQ               3.3227253 sec
RG                    90.5
DW               101.400 usec
DE                  6.00 usec
TE                 310.0 K
D1               1.00000000 sec

============ CHANNEL f1 =============
NUC1                   1H
P1                  8.30 usec
PL1                -6.00 dB
SFO1          400.1422935 MHz

F2 - Processing parameters
SI                    32768
SF               400.1400127 MHz
WDW                      EM
SSB                       0
LB                   0.30 Hz
GB                        0
PC                     1.40

1D NMR plot parameters
CX                  20.00 cm
F1P                 9.949 ppm
F1                3981.16 Hz
F2P                -0.305 ppm
F2                -121.87 Hz
PPMCM          0.51270 ppm/cm
HZCM          205.15126 Hz/cm
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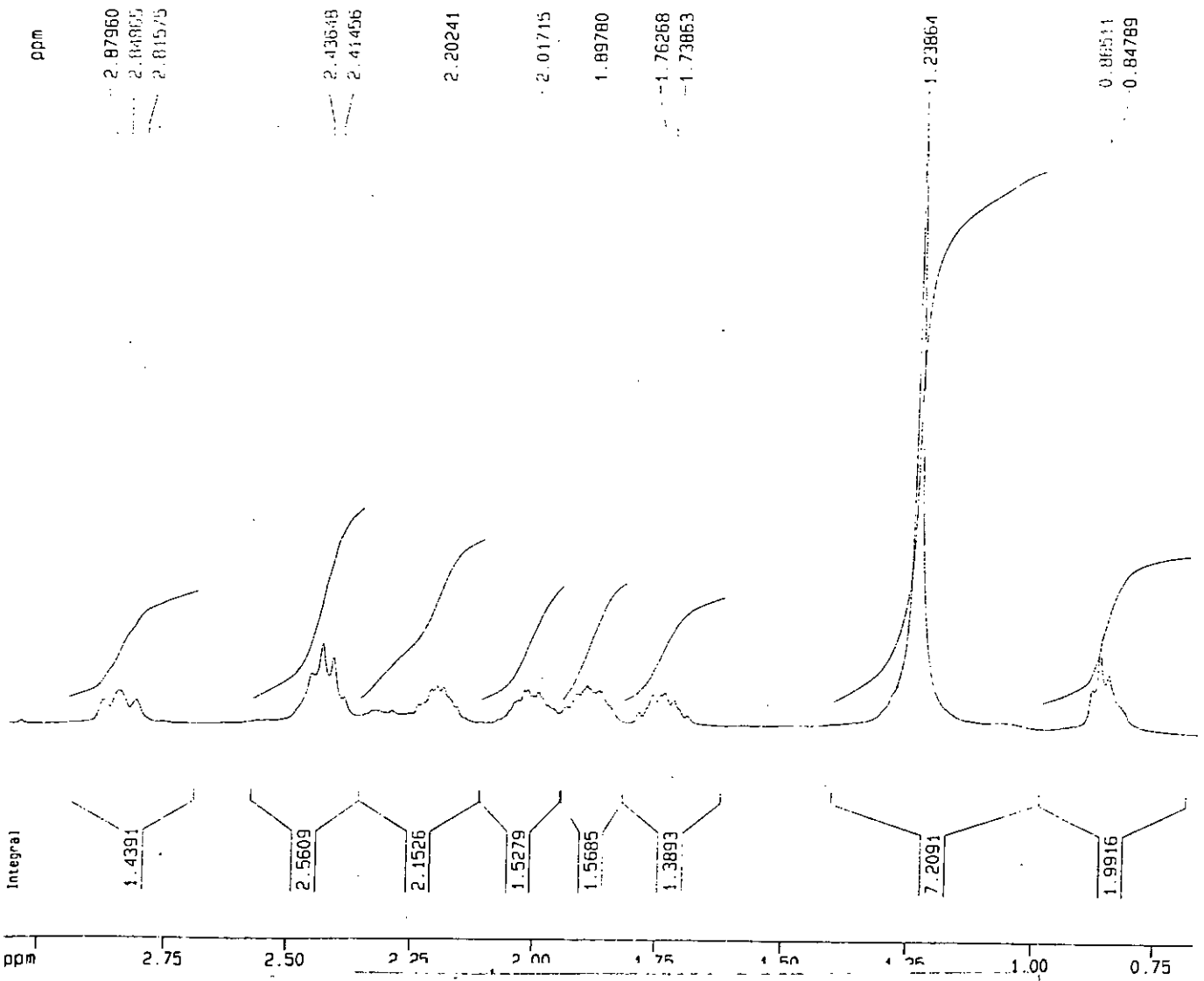


Fig.4.14b: ^{1}H- nmr spectrum of the compound A_5:

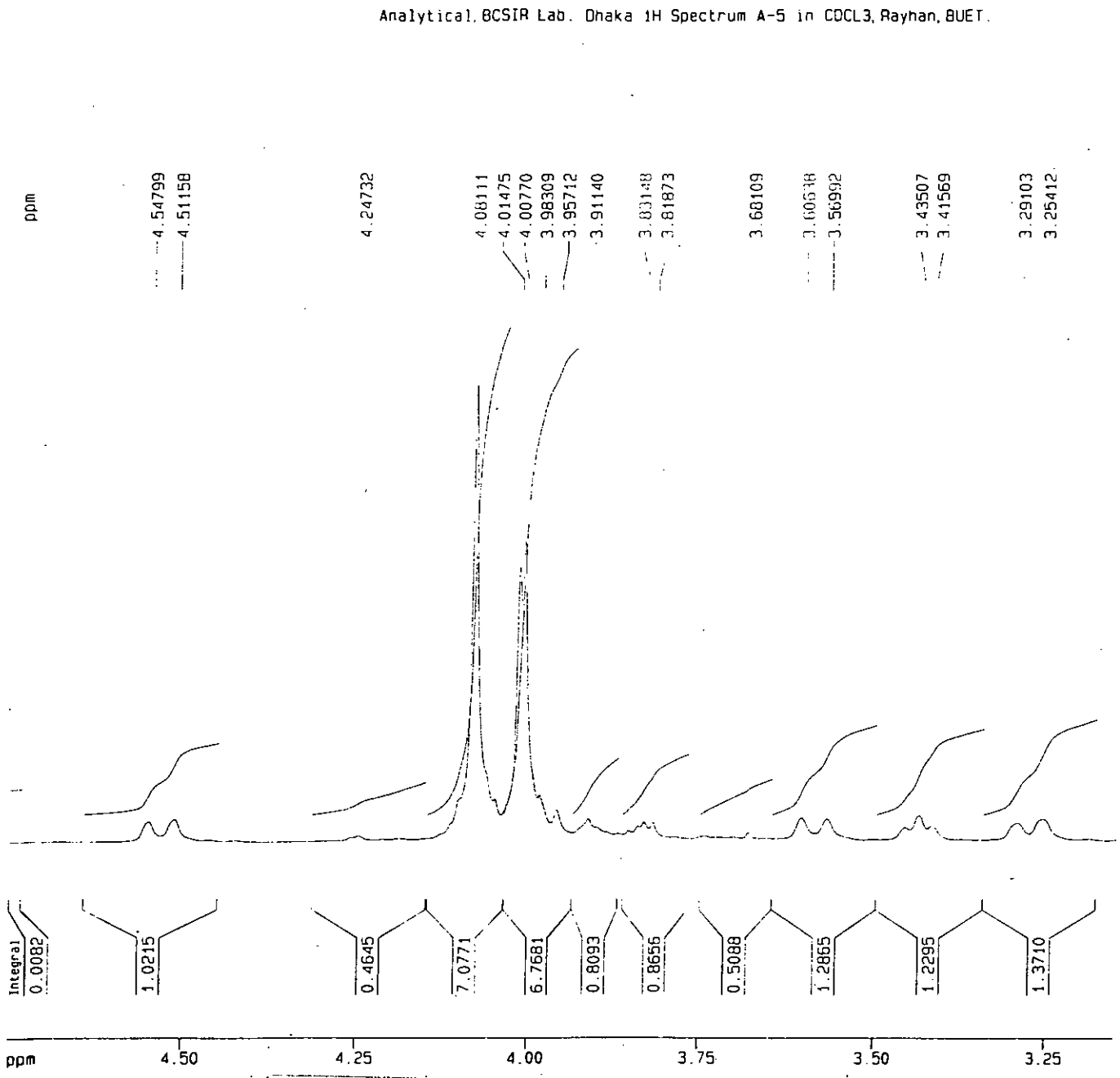
```
Current Data Parameters
NAME                  A1789
EXPNO                     1
PROCNO                    1

F2 - Acquisition Parameters
Date_              20050426
Time                  11 31
INSTRUM             qdx400
PROBHD     5 mm Multinuc
PULPROG                zg30
TD                    32768
SOLVENT               CDCl3
NS                      128
DS                        2
SWH               4930.966 Hz
FIDRES           0.150481 Hz
AQ               3.3227253 sec
RG                     90.5
DW                101.400 usec
DE                  6.00 usec
TE                 310.0 K
D1              1.00000000 sec

========== CHANNEL f1 ============
NUC1                     1H
P1                    8.30 usec
PL1                  -6.00 dB
SFO1          400.1422935 MHz

F2 - Processing parameters
SI                    32768
SF           400.1400127 MHz
WDW                      EM
SSB                       0
LB                     0.30 Hz
GB                        0
PC                     1.40

1D NMR plot parameters
CX                    20.00 cm
F1P                   3.061 ppm
F1                  1224.84 Hz
F2P                   0.665 ppm
F2                  265.90 Hz
PPMCM             0.11983 ppm/cm
HZCM             47.94723 Hz/cm
```



Current Data Parameters

NAME	A1789
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

Date_	20050426
Time	11.31
INSTRUM	dpx400
PROBHD	5 mm Multinuc
PULPROG	zg30
TD	32768
SOLVENT	CDCl3
NS	128
DS	2
SWH	4930.966 Hz
FIDRES	0.150481 Hz
AQ	3.3227253 sec
RG	90.5
DW	101.400 usec
DE	6.00 usec
TE	310.0 K
D1	1.00000000 sec

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

NUC1	1H
P1	8.30 usec
PL1	-6.00 dB
SFO1	400.1422935 MHz

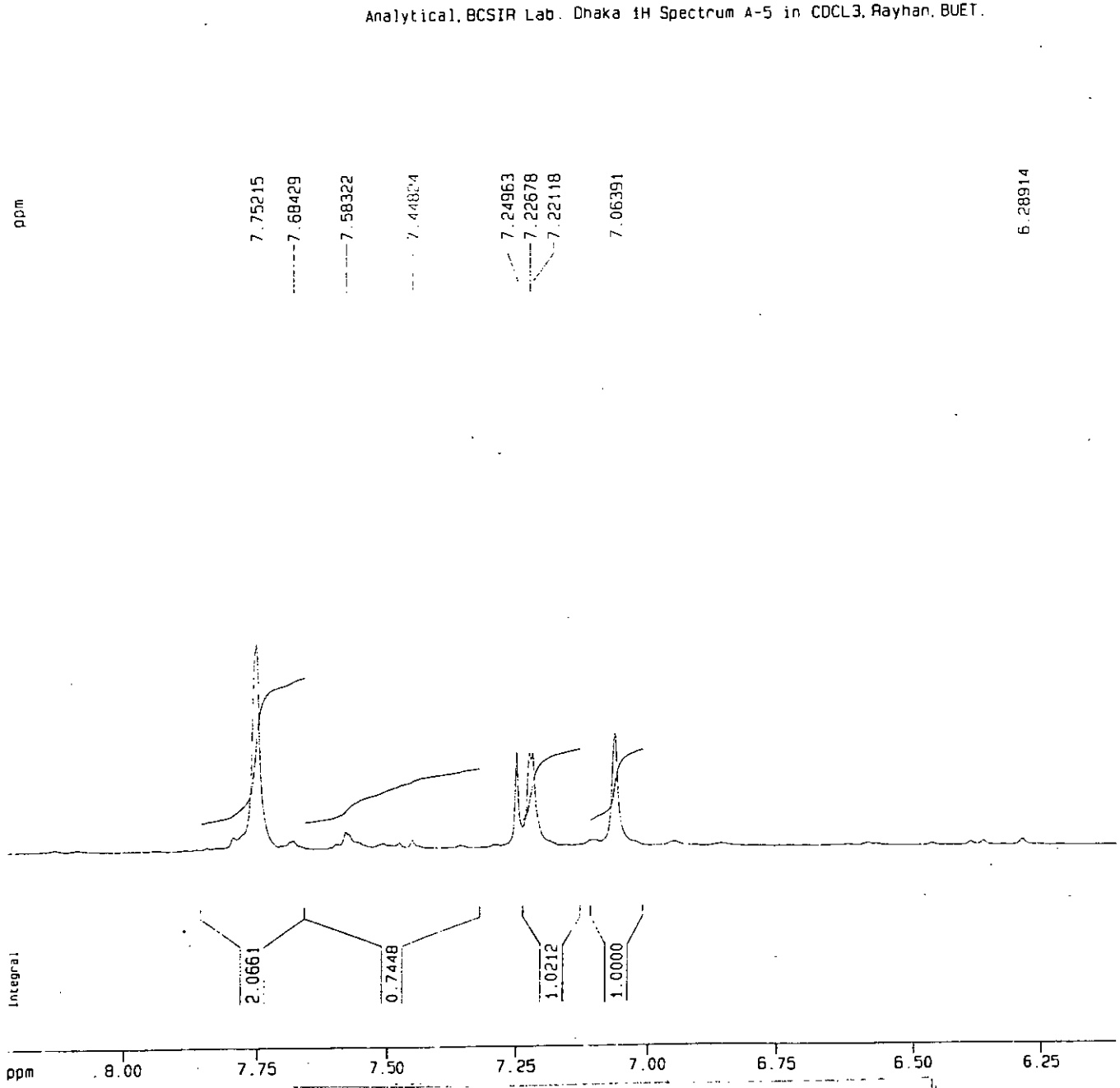
F2 - Processing parameters

SI	32768
SF	400.1400127 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.40

1D NMR plot parameters

CX	20.00 cm
F1P	4.748 ppm
F1	1899.78 Hz
F2P	3.146 ppm
F2	1258.93 Hz
PPMCM	0.08008 ppm/cm
HZCM	32.04251 Hz/cm

Fig.4.14c: ^{1}H- nmr spectrum of the compound A_5:



Current Data Parameters

NAME	A1789
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

Date_	20050426
Time	11.31
INSTRUM	dpx400
PROBHD	5 mm Multinuc
PULPROG	zg30
TD	32768
SOLVENT	CDCl3
NS	128
DS	2
SWH	4930.966 Hz
FIDRES	0.150481 Hz
AQ	3.3227253 sec
RG	90.5
DW	101.400 usec
DE	6.00 usec
TE	310.0 K
D1	1.00000000 sec

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

NUC1	1H
P1	8.30 usec
PL1	-5.00 dB
SFO1	400.1422935 MHz

F2 - Processing parameters

SI	32768
SF	400.1400127 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.40

1D NMR plot parameters

CX	20.00 cm
F1P	8.228 ppm
F1	3292.35 Hz
F2P	6.106 ppm
F2	2443.14 Hz
PPMCM	0.10611 ppm/cm
HZCM	42.46062 Hz/cm

Fig.4.14d: ^{1}H- nmr spectrum of the compound A_5:

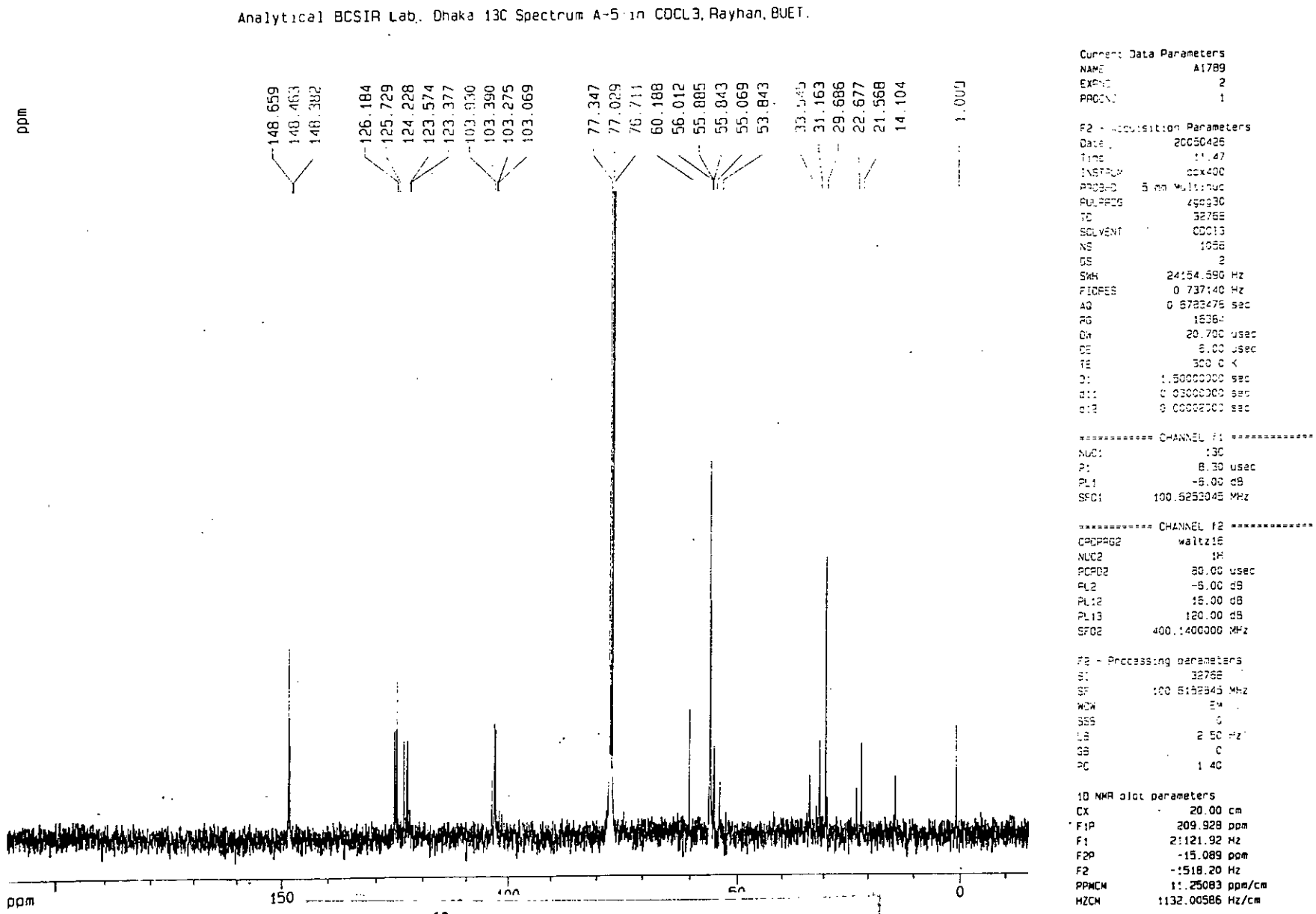
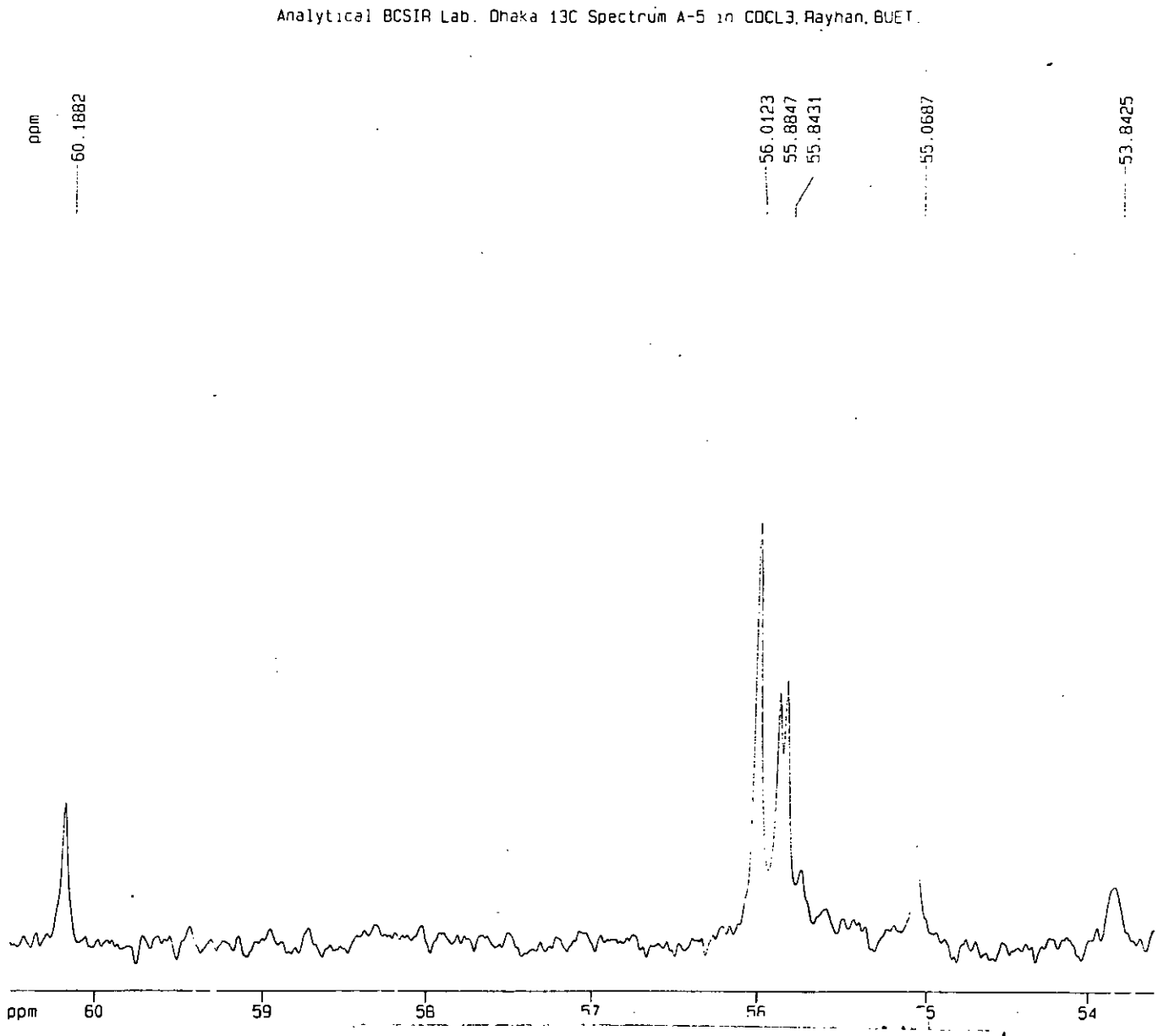


Fig.4.15a: ^{13}C- nmr spectrum of the compound A_5



Current Data Parameters
NAME A1789
EXPNO 2
PROCNO 1

F2 - Acquisition Parameters
Date_ 20050426
Time 11.47
INSTRUM spx400
PROBHD 5 mm Multinuc
PULPROG zgpg30
TD 32768
SOLVENT CDCl3
NS 1056
DS 2
SWH 24154.590 Hz
FIDRES 0.737140 Hz
AQ 0.6783475 sec
RG 16384
DW 20.700 usec
DE 6.00 usec
TE 300.0 K
D1 1.50000000 sec
d11 0.03000000 sec
d12 0.00002000 sec

========== CHANNEL f1 ==========
NUC1 13C
P1 8.30 usec
PL1 -5.00 dB
SFO1 100.6253045 MHz

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

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

1D NMR plot parameters
CX 20.00 cm
F1P 60.513 ppm
F1 6088.50 Hz
F2P 53.597 ppm
F2 5392.73 Hz
PPMCM 0.34576 ppm/cm
HZCM 34.78874 Hz/cm

Fig.4.15b: ^{13}C- nmr spectrum of the compound A_5.

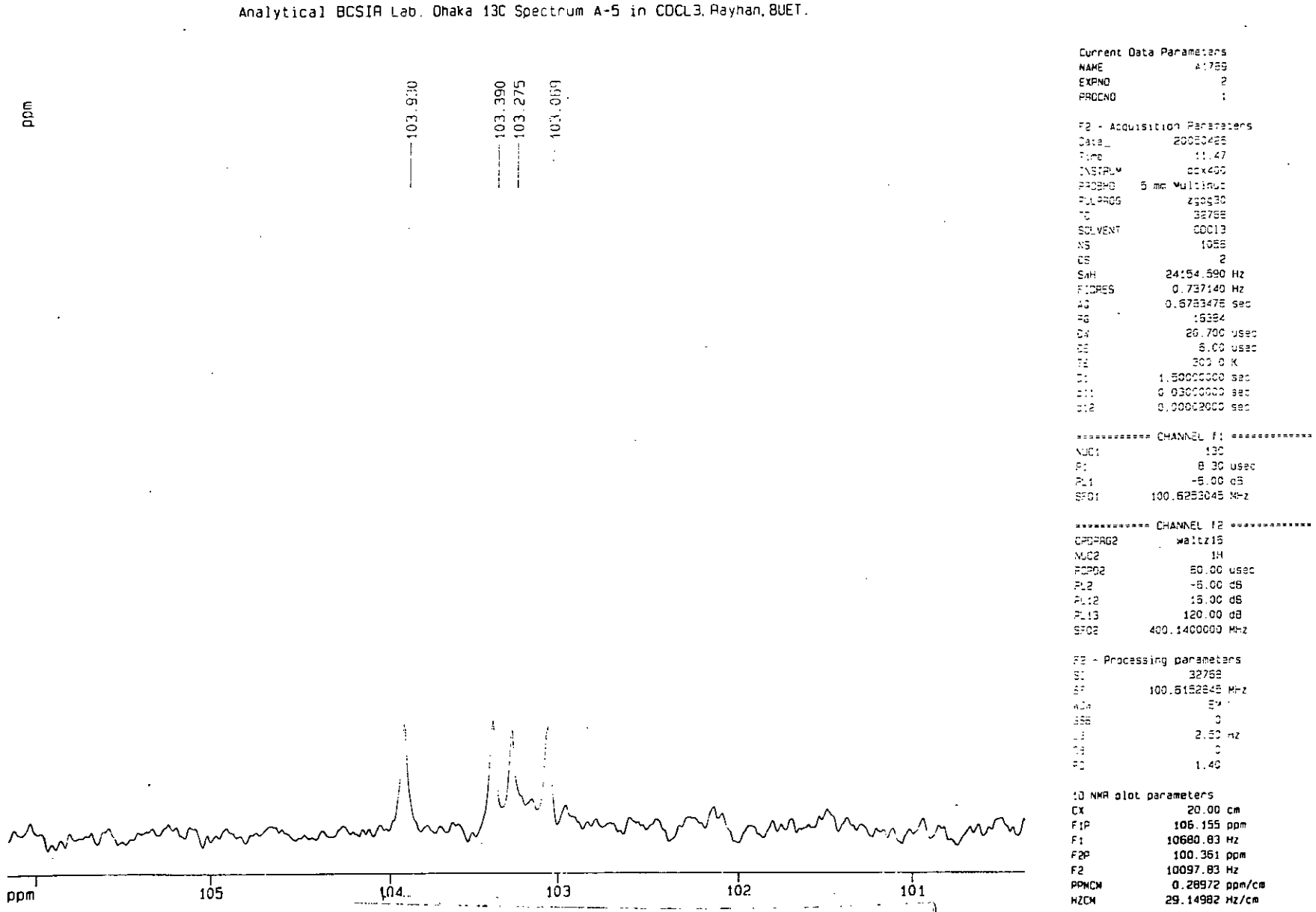
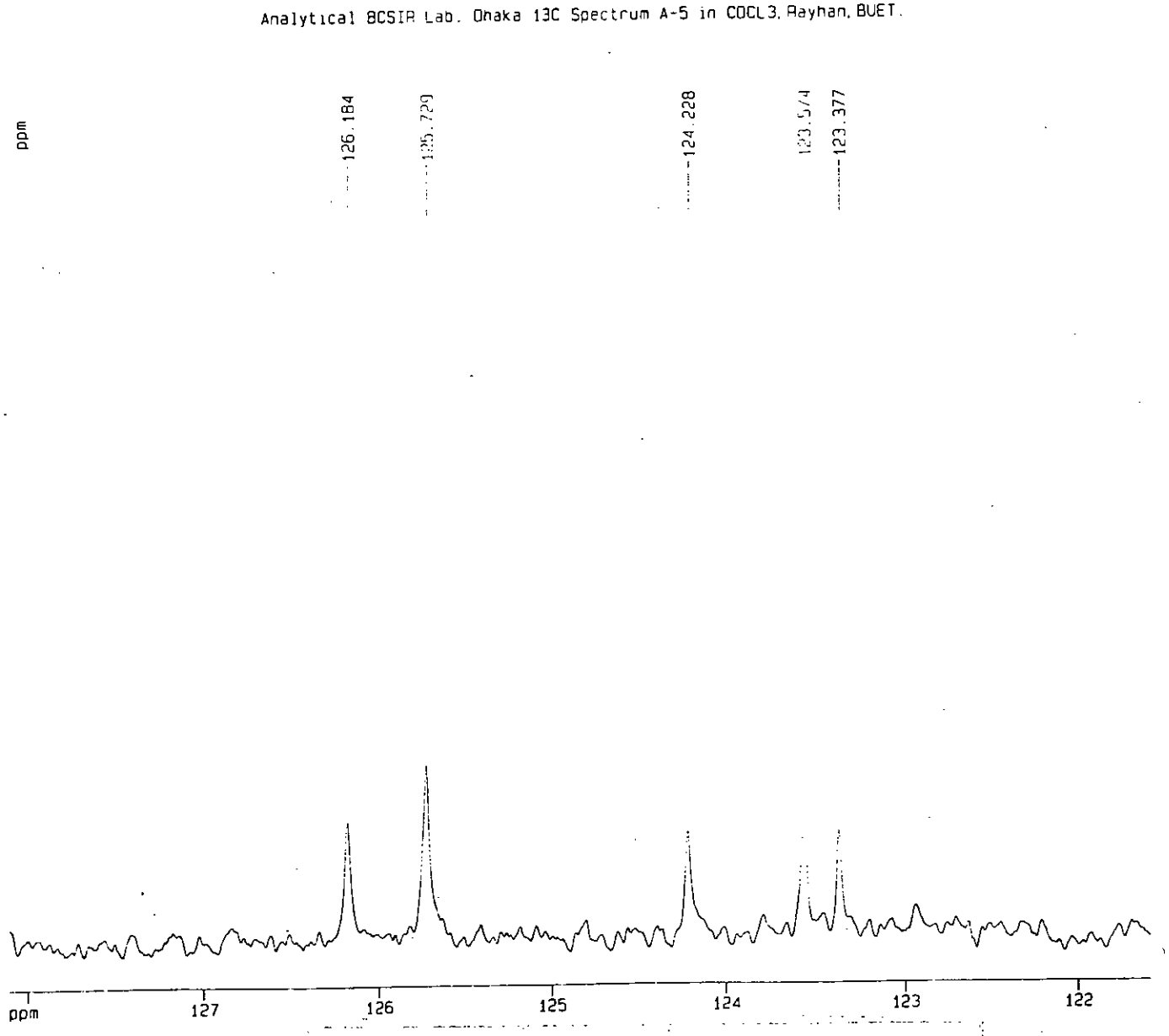


Fig.4.15c: ^{13}C- nmr spectrum of the compound A_5:



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EXPNO 2
PROCNO 1

F2 - Acquisition Parameters
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PROBHD 5 mm multinuc
PULPROG zgpg30
TD 32768
SOLVENT CDCl3
NS 1058
DS 2
SWH 24154.589 Hz
FIDRES 0.737140 Hz
AQ 0.5783473 sec
RG 16384
DW 20.700 usec
DE 6.00 usec
TE 300.0 K
D1 1.50000000 sec
D11 0.03000000 sec
D12 0.00002000 sec

========== CHANNEL f1 ==========
NUC1 13C
P1 8.30 usec
PL1 -6.00 dB
SFO1 100.6253048 MHz

========== CHANNEL f2 ==========
CPDPRG2 waltz16
NUC2 1H
PCPD2 20.00 usec
PL2 -6.00 dB
PL12 15.00 dB
PL13 120.00 dB
SFO2 400.1400000 MHz

F2 - Processing parameters
SI 32768
SF 100.6152840 MHz
WDW EM
SSB 0
LB 2.50 Hz
GB 0
PC 1.40

1D NMR plot parameters
CX 20.00 cm
F1P 126.109 ppm
F1 12689.73 Hz
F2P 121.578 ppm
F2 12232.59 Hz
PPMCM 0.32656 ppm/cm
HZCM 32.85695 Hz/cm

Fig.4.15d: ^{13}C- nmr spectrum of the compound A_5:

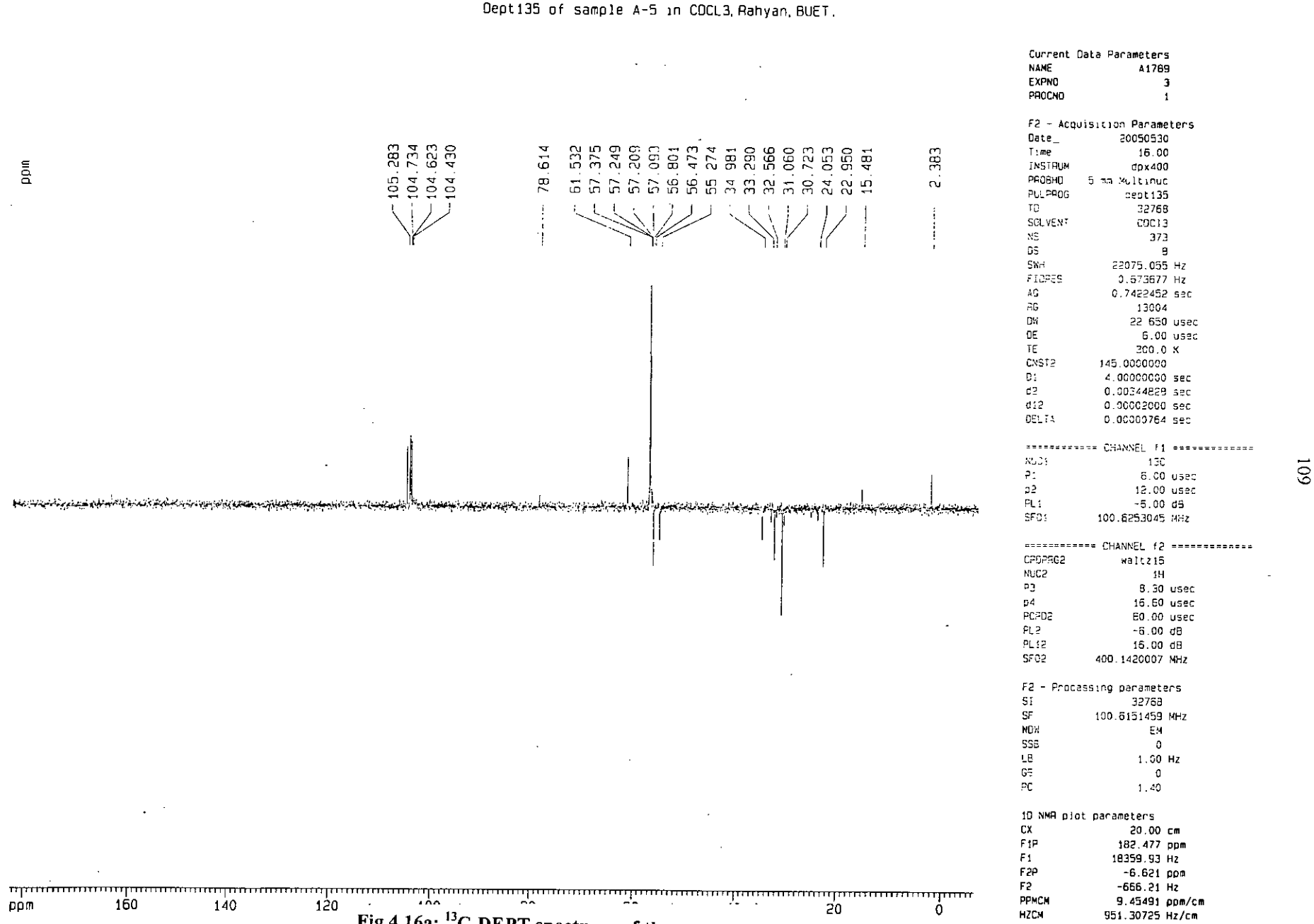

Fig 4.16a: ^{13}C-DEPT spectrum of the compound A_5:

```
Current Data Parameters
NAME                  A1789
EXPNO                     3
PROCNO                    1

F2 - Acquisition Parameters
Date_              20050530
Time                  16.00
INSTRUM               dpx400
PROBHD     5 mm Multinuc
PULPROG             dept135
TD                    32768
SOLVENT               CDCl3
NS                      373
DS                        8
SWH               22075.055 Hz
FIDRES            0.673677 Hz
AQ                0.7422452 sec
RG                    13004
DW                 22.650 usec
DE                  6.00 usec
TE                  300.0 K
CNST2             145.0000000
D1                4.00000000 sec
d2                0.00344828 sec
d12               0.00002000 sec
DELTA             0.00000764 sec

============ CHANNEL f1 =============
NUC1                    13C
P1                   6.00 usec
p2                  12.00 usec
PL1                 -5.00 dB
SFO1           100.6253045 MHz

============ CHANNEL f2 =============
CPDPRG2             waltz16
NUC2                    1H
P3                   8.30 usec
p4                  16.60 usec
PCPD2               80.00 usec
PL2                 -6.00 dB
PL12                15.00 dB
SFO2           400.1420007 MHz

F2 - Processing parameters
SI                    32768
SF             100.6151459 MHz
WDW                     EM
SSB                      0
LB                    1.00 Hz
GB                       0
PC                    1.40

1D NMR plot parameters
CX                   20.00 cm
F1P                182.477 ppm
F1                 18359.93 Hz
F2P                 -6.621 ppm
F2                 -666.21 Hz
PPMCM               9.45491 ppm/cm
HZCM              951.30725 Hz/cm
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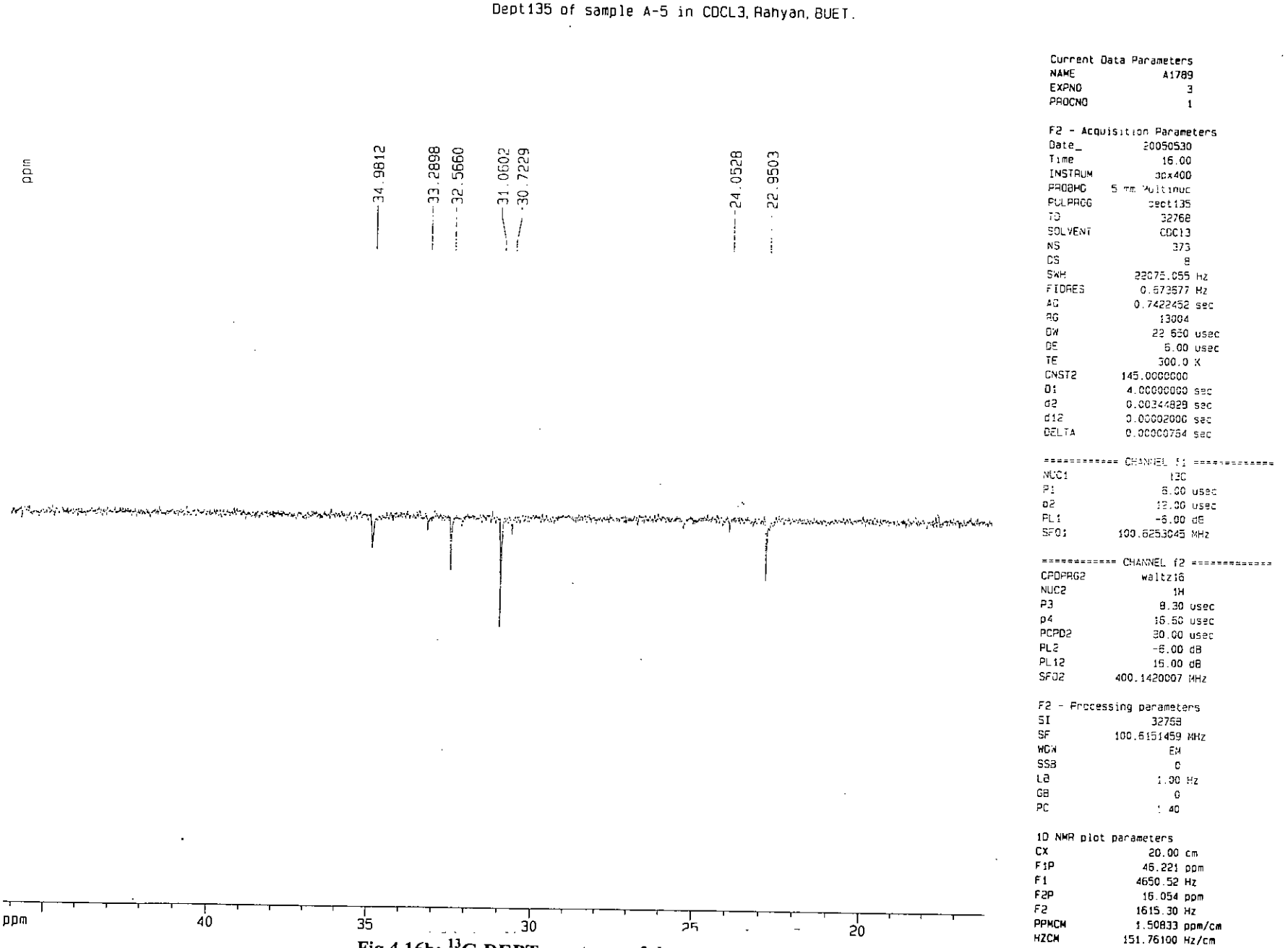


Fig 4.16b: ^{13}C-DEPT spectrum of the compound A_5:

Current Data Parameters

NAME	A1789
EXPNO	3
PROCNO	1

F2 - Acquisition Parameters

Date_	20050530
Time	16.00
INSTRUM	300x400
PROBHD	5 mm Multinuc
PULPROG	dept135
TD	32768
SOLVENT	CDCl3
NS	373
DS	8
SWH	22075.055 Hz
FIDRES	0.673577 Hz
AQ	0.7422452 sec
RG	13004
DW	22.650 usec
DE	6.00 usec
TE	300.0 K
CNST2	145.0000000
D1	4.00000000 sec
d2	0.00344829 sec
d12	0.00002000 sec
DELTA	0.00000754 sec

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

NUC1	13C
P1	8.00 usec
p2	16.00 usec
PL1	-6.00 dB
SFO1	100.6253045 MHz

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

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

F2 - Processing parameters

SI	32768
SF	100.6151459 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	1.40

1D NMR plot parameters

CX	20.00 cm
F1P	46.221 ppm
F1	4650.52 Hz
F2P	16.054 ppm
F2	1615.30 Hz
PPMCM	1.50833 ppm/cm
HZCM	151.76100 Hz/cm

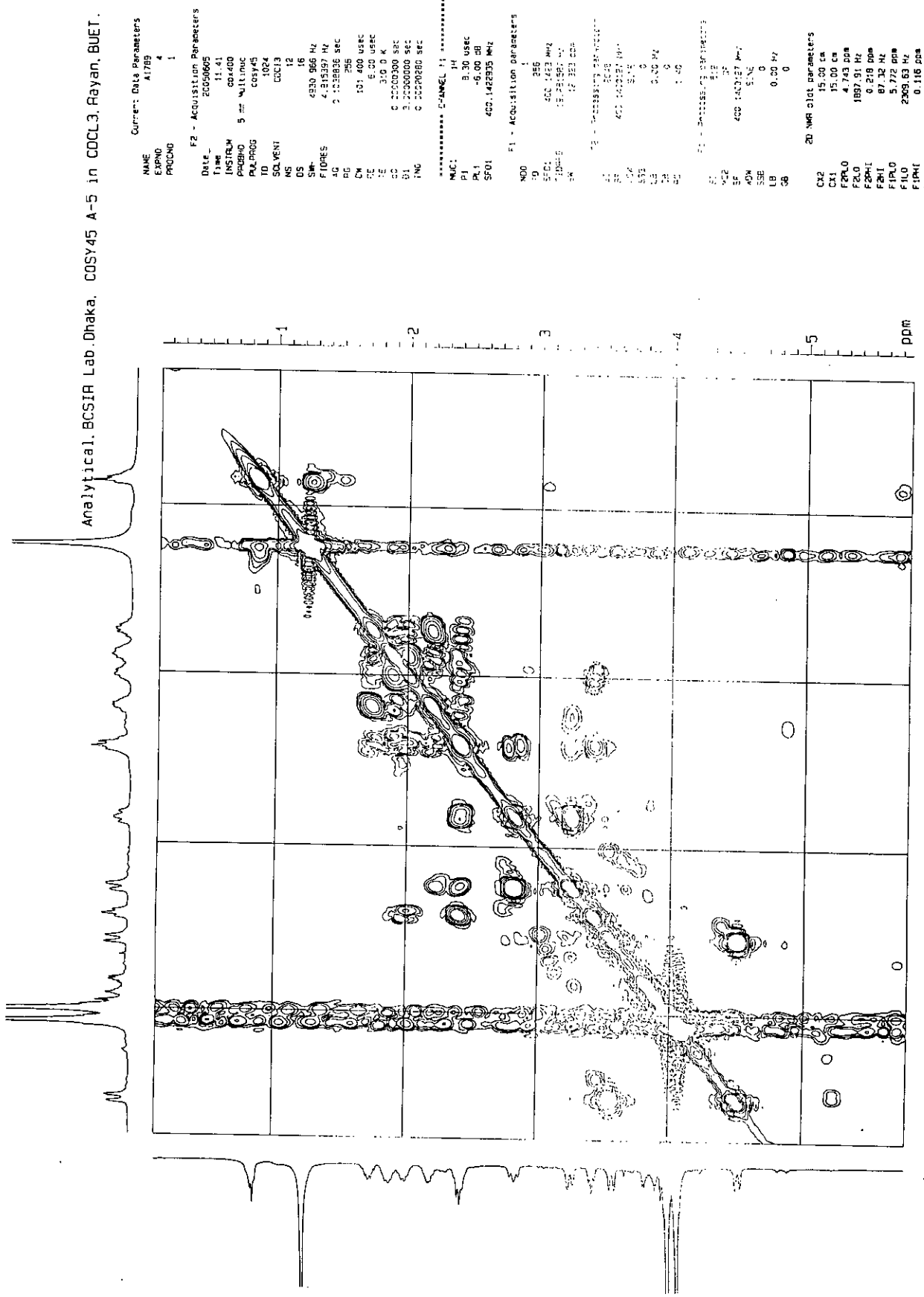
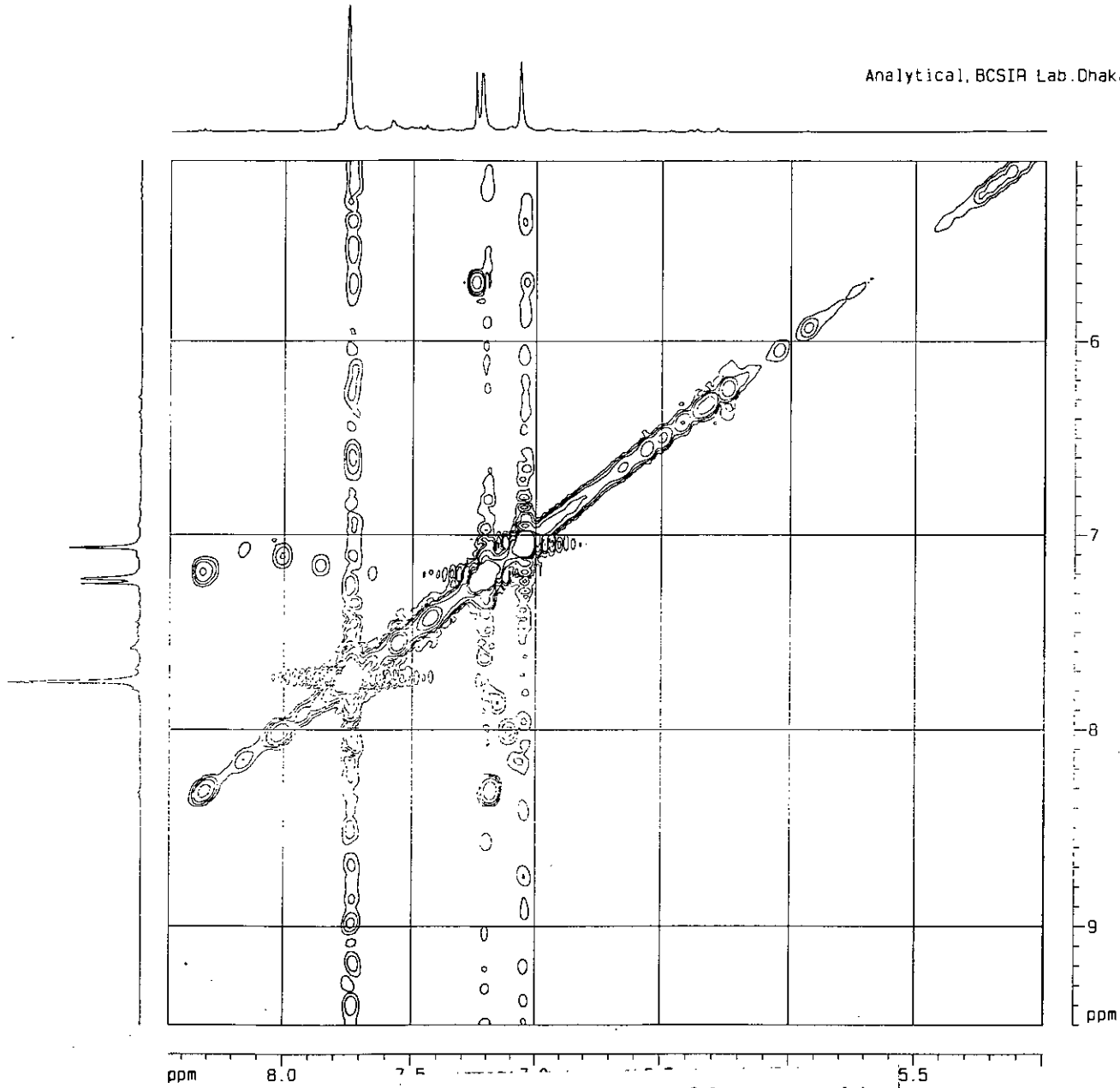


Fig.4.17a:COSY spectrum of the compound A_5:



Current Data Parameters

NAME	A1789
EXPNO	4
PROCNO	1

F2 - Acquisition Parameters

Date_	20050605
Time	11.41
INSTRUM	dpx400
PROBHD	5 mm Multinuc
PULPROG	cosy45
TD	1024
SOLVENT	CDCl3
NS	12
DS	16
SWH	4930.966 Hz
FIDRES	4.815397 Hz
AQ	0.1038836 sec
RG	256
DW	101.400 usec
DE	6.00 usec
TE	310.2 K
d0	0.00000300 sec
D1	1.00000000 sec
INO	0.00020280 sec

********** CHANNEL f1 **********

NUC1	1H
P1	8.30 usec
PL1	-6.00 dB
SFO1	400.1422935 MHz

F1 - Acquisition parameters

NDO	1
TD	256
SFO1	400.1423 MHz
FIDRES	19.26158 Hz
SW	12.373 ppm

F2 - Processing parameters

SI	2048
SF	400.1400000 MHz
WDW	QSINE
SSB	1
LB	0.00 Hz
GB	0
PC	1.40

F1 - Processing parameters

SI	512
MC2	QF
SF	400.1400000 MHz
WDW	SINE
SSB	0
LB	0.00 Hz
GB	0

2D NMR plot parameters

CX2	15.00 cm
CX1	15.00 cm
F2PLO	8.456 ppm
F2LO	3383.46 Hz
F2PHI	4.996 ppm
F2HI	1998.03 Hz
F1PLO	9.503 ppm
F1LO	3802.40 Hz
F1PHI	5.074 ppm
F1HI	2030.33 Hz
F2PPMCM	0.23066 ppm/cm
F2HZCM	92.29511 Hz/cm
F1PPMCM	0.29524 ppm/cm
F1HZCM	118.13774 Hz/cm

Fig.4.17b:COSY spectrum of the compound A_5:

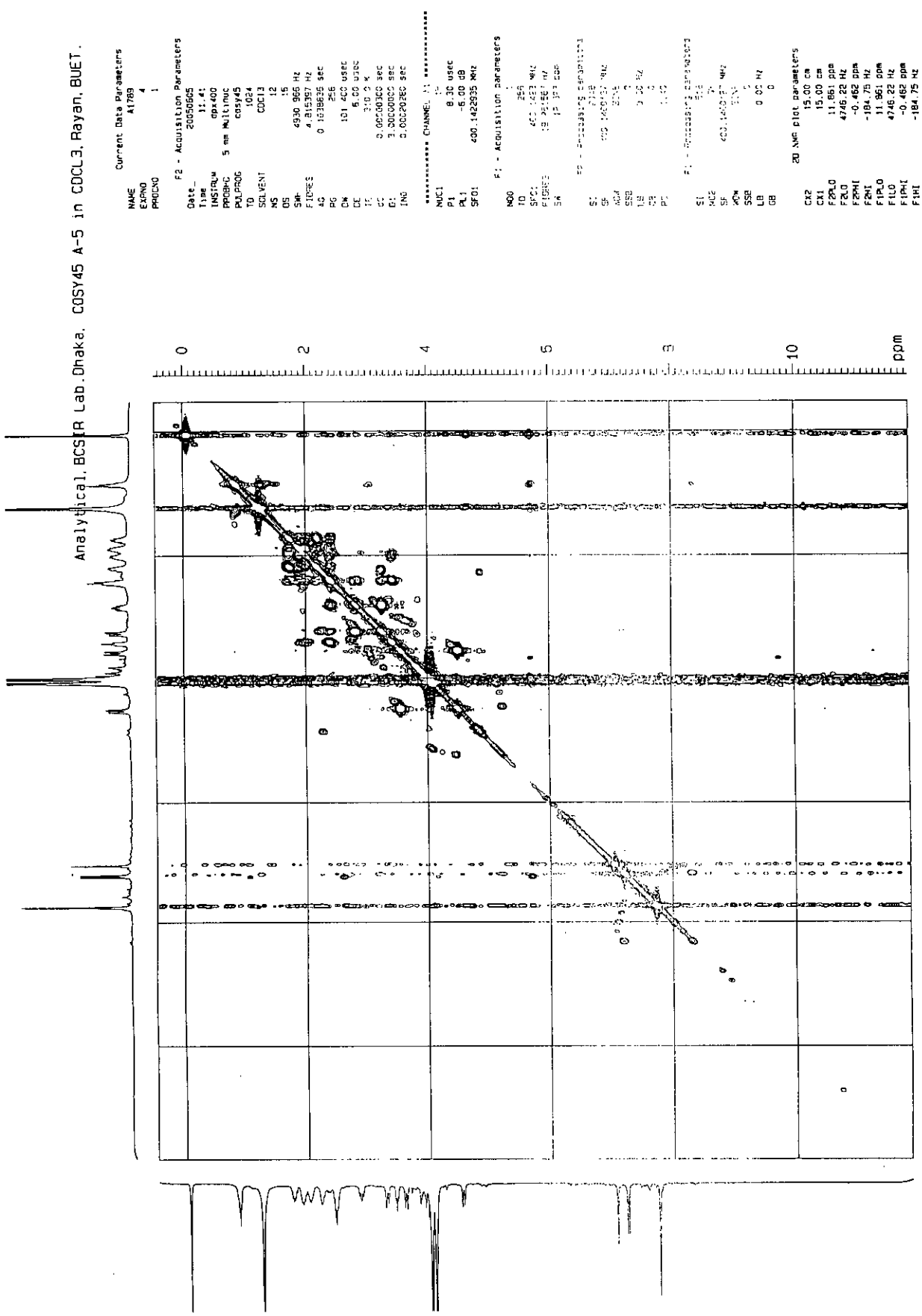


Fig.4.17c:COSY spectrum of the compound A_5:

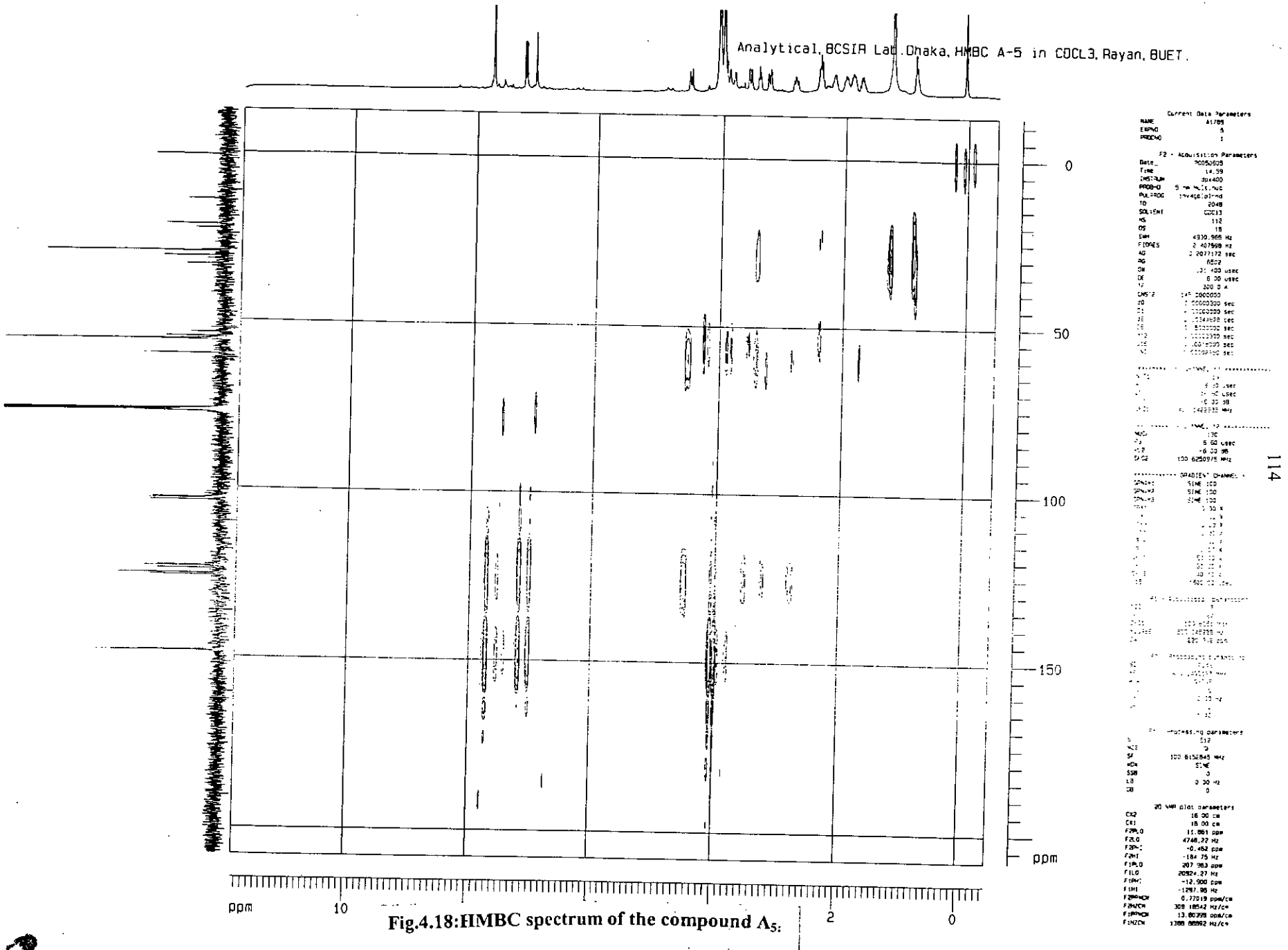


Fig.4.18:HMBC spectrum of the compound A_5:

5.0: CHAPTER 5

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