# PHYTOCHEMICAL INVESTIGATION ON OYSTER MUSHROOM

## *(Pleurotus ostreatus)*



THESIS PRESENTED

BY

HASINA BEGOM BINA

FOR

THE DEGREE OF MASTER OF PHILOSOPHY

OF

THE UNIVERSITY OF DHAKA

EXAMINATION ROLL NO. 10

REGISTRATION NO. 214

SESSION. 2002-03



DEPARTMENT OF CHEMISTRY

THE UNIVERSITY OF DHAKA

DHAKA-1000

MARCH 2009

22/3/09

Dr. Totail Ahmad Chowdhury
Professor, Dept. of Chemistry
Dhaka University, Dhaka-1000



22.3.09

(Prof. S.M. Mizanur Rahman)

Co-supervisor

D. S. M. Mizanur Rahman
Professor
Dept. of Chemistry, D.U.
Bangladesh.

**DEDICATED**

**TO**

**MY BELOVED PARENTS**

***JAHANARA AZAD BAKUL***

***&***

***ABUL KALAM AZAD***

# ACKNOWLEDGEMENT

First of all I am grateful to almighty Allah for empowering me to complete the research work and also submission of the thesis.

It is my great pleasure to express my best regards, profound gratitude and deep appreciation to my reverend supervisor Dr. Tofail Ahmad Chowdhury, Professor, Department of Chemistry, University of Dhaka, for his constant supervision, affectionate encouragement, valuable discussion and suggestions through out the entire period of my research work.

I also gratefully acknowledge my indebtness to Dr. S.M. Mizunur Rahman, Professor, Department of Chemistry, University of Dhaka, for his constant supervision, affectionate inspiration and guidance during the progress of the research work.

I would like to express my heartfelt gratitude to Dr. Md. Tanvir Muslim, Associate Professor, Department of Chemistry, University of Dhaka, for his affectionate inspiration and cooperation.

I am also expressing my special thanks to all M.Phil. and Ph.D. researchers for their help at different stages of my research work.

I also acknowledge the support made by technical officer Md. Akram Hossain, and lab atteddants Md. Malek, and Md. Emdad Hossain, during the progress of research work.



I cannot find adequate words for expressing my love and gratitude to my sisters, brothers, nephews, Afrin, Sharmin and Nila for their moral support and companionship during the research work.

I am also thankful to all of my friends.

March, 2009

Author

**HASINA BEGOM BINA**
DEPARTMENT OF CHEMISTRY
UNIVERSITY OF DHAKA

# CONTENTS

**2. METHODOLOGY** **PAGE NO.**

**3 EXPERIMENTAL** **PAGE NO.**

**4. ANALYSIS OF ASH, MOISTURE, ACID VALUE, SAPONIFICATION VALUE, IODINE VALUD AND FATTY ACIDS**

**PAGE NO.**

**LISTS OF TABLES**

**LIST OF FIGURES**

**LIST OF SCHEMES**

## AIM OF THE PRESENT WORK

Mushrooms are one type of fungi. It grows in tropical climate. In Bangladesh it grows wild all over the country under favorable climatic conditions. Most of the mushrooms have nutritional and medicinal value. Mushrooms contain 19%-55% protein on dry weight basis. Scientific reports on their antitumor activity, antiinflammatory activity, antimicrobial activity, antiviral activity and gene protective activity are available. It also offers antidiabetic, anticancer, antioxidant and anticardiovascular properties. Apart from above medicinal applications now-a-days mushrooms are also used in different brands of cosmetic products, soaps, creame, toothpaste, ice creame, beverages and also in food decoration.

Oyster mushroom (*Pleurotus ostreatus)* belongs to the pleurotaceae family, is an edible mushroom used as nutritious and delicious food. Now-a days it is cultivated in many places of the country. Literature reports on *Pleurotus ostreatus* indicates that it is rich in protein and its medicinal value is also well known. To study the medicinal importance of *Pleurotus ostreatus,* it was planned to make a systemstic chemical investigation. This investigation will include the determination of the proximate composition such as, dry matter, moisture, ash etc.

Extractions of the powdered mushroom will be carried out using different solvents of varying polarity. These extracts will be investigated by thin layer chromatography and paper chromatography and also will be subjected to different color reactions for detecting different class of organic compounds (alkaloids, terpenoids, flavonoids, steroids, carbohydrates.) Different chromatographic techniques (column chromatography, vaccum liquid chromatography etc.) will be applied to isolate pure compound(s) from the extracts. The structure of the isolated compounds will be elucidated using chemical and spectroscopic techniques.

Non polar extracts (n-hexane) extract will be subjected to analysis for its fatty matter (fat, lipid). Identification and quantification of the constituent carboxylic acids present in the fat will be made using gas liquid chromatography.

# 1. INTRODUCTION

# 1. INTRODUCTION

## 1.1 GENERAL

Mushrooms belong to the fungi family. Although most fungi have plant-like cells, but they miss the most important feature of plants chlorophyll. Chlorophyll enables plants to produce food directly from carbon dioxide and water using the energy from sun. Fungi, therefore grows only where there is organic material or a host to feed off, in the process decomposing organic material left behind by plants and animals. This plays an important role in breaking down organic wastes such as cellulose or lignin to make them available to other organisms and plants. Many by-products from agricultural production and food processing, for example, can be used as growing material in mushroom production.

Edible mushrooms are very nutritious. Mushrooms grow naturally on the trunks, leaves and roots of trees as well as decaying woody materials (Chang and Miles, 1992; Stamets, 2000; Linde-quist *et al.*, 2005). Mushrooms have shown that they serve as repositories of B-vitamins such as niacin, flavin and pyridoxine (Solomko and Eliseeva, 1988); organic acids such as ascorbic, shikimic, malic and fumaric; carbohydrates such as the β-glucans; monoterpenoid and diterpenoid lipids; proteins such as hydrophobins and trace elements such as selenium (Dikeman *et al.*, 2005; Valentao *et al.*, 2005). These substances are responsible for the antimicrobial, antioxidant, antitumor, antihypertensive and antiaging potentials of edible mushrooms (Lindequist *et al.*, 2005). Antioxidant mushrooms are due to its phenolic, terpenoids and polysaccharide polypeptide contents (Miller *et al.*, 2000; Mau *et al.*, 2002; Valentao *et al.*, 2005).

Oyster mushroom *(Pleurotus ostreatus)* exibit antibacterial and antifungal[1-5] activity . Recent studies on various Pleurotus species have shown a number of therapeutic activities, such as antitumour, immunomodulatory, antigenotoxic, antioxidant, antiinflammatory, hypocholesterolaemic, antihypertensive, antiplatelet-aggregating, antihyperglycaemic, antimicrobial and antiviral activities.

The present nutritional status of Bangladesh is a matter of great concern. Wide spread malnutrition with ever increasing protein gap with inhibitory diet against complicated human diseases in the country necessitates the search for alternative source of protein. . Mushroom can produce highest quantity of protein per unit area (Pathak, *el al.* 1998) Mushrooms can reduce the suffering from malnutrition to some extent.

.

Mushroom was introduced in Bangladesh in 1979 through Department of Agricultural Extension (DAE). Since 1979 DAE has been involved in its routine production using the technology adopted from developed countries like Japan. They cultivates three kinds of mushrooms. These are straw mushroom (*Volvuriella volvacea*), oyster mushroom (*Pleurotus ostreatus*) and jaw ear mushroom (*Auncularia polytrichan*). Among the five oyster mushroom species, available in Mushroom Culture Center (MCC), Savar, *Pleurotus ostreatus* gave the highest yield (56.33 g/500g spawn packet) of first flush of harvest and took the shortest time for mycelium running in test tube and lowest harvesting time (Amin, 2002).

Trivial people living at the hilly area consume mushrooms from ancient time because at present there is a large demand of mushroom in local market. DAE can supply only 5%-6% of our local demand. The rest of the demand of the mushroom is fulfilled by importing dried or canned mushroom. As the environment of Bangladesh is most favorable, for the cultivation of mushroom and owing to its high local demand as well as export potentialities a number of private growers are interested for its cultivation. But due to high production cost most of the growers are losing their interest to its cultivation. Therefore, it is necessary to develop low cost and high yielding production technology. Now-a-days the private sector started to develop mushroom cultivation in Bangladesh to meet local demand and also create opportunities for export.

## 1.2 BOTANICAL CHARACTERISTICS OF THE *PLEUROTUS OSTREATUS*

**Cap** (pileus) 1 - 12 inches wide, 0.5 to 1.5 inches thick. Convex and semicircular to fan shaped, overlapping in large bunches. Either species usually has a strong anise-like aroma. Older fruit bodies may develop some off aromas. *Pleurotus ostreatus* cap color is likely to be tan to brown but can be whitish, grayish or even gray-blue. The gray-blue color is more likely to be seen in cultivated *P. ostreatus* of European origin. *Pleurotus populinus* has a whitish ivory cap.

**Gills** (lamellae) Fairly close together running down the stem and white, light gray or tannish. *Pleurotus populinus* is likely to be whitish ivory. *P. ostreatus* will have more variation in color being usually tan to brown.

**Stem** (stipe) Usually nonexistent to stubby unless growing on top of a log and bunched together (cespitose).

**Flesh** Thick, white and non bruising.

**Spores** *P. ostreatus* makes a whitish gray or lavender/lilac spore print. The thicker the spore print is, the more likely some color will show. *P. populinus* makes a white to very slightly grayish spore print.

*Fig 1.1 Pleurotus ostreatus*

## 1.3 CULTIVATION OF MUSHROOM

Temperature plays a vital role in the agronomic condition of mushroom cultivation. Recommended conditions for mushroom cultivation[6-11] are temperature, luminosity, precocity, yield, pileus area, stalk formation pattern, coloration and handling resistance. Effects of temperature (15°C and 28°C) and luminosity (120 and 900 lux) were evaluated for eight *P. ostreatus* strains in relation to precocity, yield, pileus area, stalk formation pattern, coloration and handling resistance. Genetic variability of strains was analysed by the Random Amplified Polymorphic DNA (RAPD) method. The Pos 98/37 strain was the only to yield white pileus at 28°C and 900 lux, and grey ones at 15°C and 120 lux. The Pos 96/05 strain, the latest, produced lead-coloured pileus at 15°C, as did the remaining strains at this temperature. Strains cultivated at 15°C did not differ in relation to handling resistance. At 28°C mushrooms were less resistant. In relation to yield, the Pos 98/38 strain was significantly more efficient. The Pos 98/37 strain, at 28°C, as compared to the same strain at 15°C, was more efficient and had an asymmetric stalk formation pattern. Among strains cultivated at 15°C, the stalk formation pattern was symmetric, except for the Pos 97/15 and Pos 97/17 strains. Molecular characterization of the Pos 98/37 strain was 30% similar to the remaining strains. The temperature of fructification and luminosity influence the induction and development of the isolates.

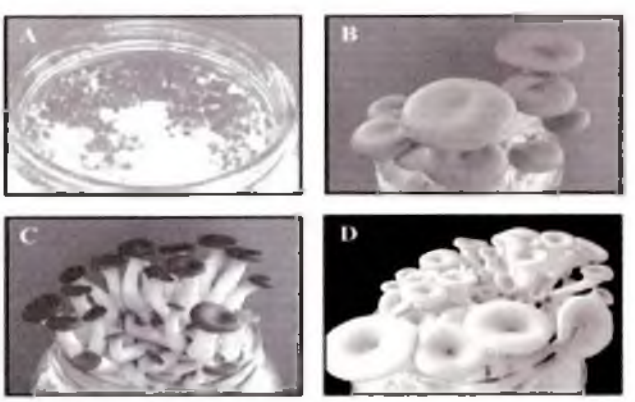



Fig. 1.2 - *P. ostreatus* basidiocarps in production conditions. A - Isolated Pos 96/05 15°C, 120 lux, after 11 days of the hydration. B - Isolated Pos 98/38 15°C, 120 lux, after 7 days of the hydration. C - Isolated Pos 98/37 15°C, 120 lux, after 11 days of hydration. D - Isolated 98/37 Pos 28°C, 900 lux, after 7 day of the hydration

**Fig:1.3 Cultivation of Mushroom**

Pleurotus mushrooms are the second most important mushrooms in production in the world, 25% of total world production of cultivated mushrooms. Pleurotus mushrooms are world-wide, China is the major producer. Several species can be grown on carbonaceous matter such as straw or newspaper. In the wild they are usually found growing on wood.

## 1.4 THE TAXONOMY OF *PLEUROTUS OSTREATUS*

*Pleurotus ostreatus* (Jacq.)Quelet[12]

*Taxonomy ID:* 5322

*Genbank common name:* oyster mushroom

*Rank:* species

*Genetic code:* Translation table 1 (Standard)

*Mitochondrial genetic code:* Translation table 4 (Mold Mitochondrial; Protozoan Mitochondrial; Coelenterate Mitochondrial; Mycoplasma; Spiroplasma)

*Lineage (full)*

**Superkingdom:** Eukaryota

**Kingdom:** Fungi

**Subkingdom:** Dikarya

**Phylum:** Basidiomycota

**Class:** Agaricomycetes

**Subclass:** Agaricomycetidae

**Order:** Agaricales

**Family:** Pleurotaceae

**Genus:** Pleurotus

**Species:** *Pleurotus ostreatus*

## 1.5 THE FAMILY OF PLEUROTACEAE

**Genus: *Pleurotus***

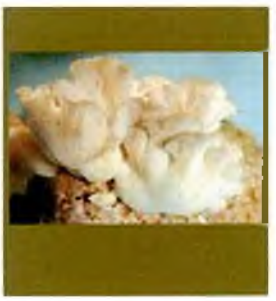
A Fleshy to tough fan-shaped fungi sessile or with stripe, colours dull or absent. There are a number of species in New Zealand, which was very difficult to identify. Some of them are considered as edible.

Species: *Pleurotus djamor*

| | |
|---|---|
| Common Name: None | Height: 60 mm |
| Substrate: Wood (Cabbage tree) | Width: 150 mm |
| Found: Mixed Forest | Season: Autumn |
| Spore: White | Edible: Yes |

Species: *Pleurotus pulmonariuss*

This is the Oyster sp. is normally found in home growing kits.



| | |
|---|---|
| Common Name: Oyster Mushroom | Height: 60 mm |
| Substrate: Straw | Width: 150 mm |
| Spore: White | Edible: Yes |

Species: Pleurotus purpureo-olivaceus (Stevenson) Segedin

| | |
|---|---|
| Common Name: None | Height: 60 mm |
| Substrate: Wood | Width: 150 mm |
| Found: Nothofagus Forest | Season: Autumn |
| Spore: White | Edible: Yes |

**Genus: Hohenbuehelia**

These saprobic fungi are similar to *Pleurotus* in appearance but much smaller with crowded gills and white spores. They also have a gelatinous layer beneath the cap which makes them quite tough to brake. Distinguished microscopically by the presence of metuloids on the gills face often encrusted with crystals.

Species: *Hohenbuehelia petalodes*

Common Name: None
Found: Mixed Forest
Substrate: Wood
Spore: White

Height: 20 mm
Width: 30 mm
Season: Autumn
Edible: Unknown

## Unidentified

The fungi below those are not identified beyond there genus. This may be miss understanding the keys or a species that has not yet been described (named).

Species: *Hohenbuehelia sp.*

Common Name: None
Found: Urban scrub
Substrate: Tree fern
Spore: White

Height: 10 mm
Width: 10 mm
Season: Autumn
Edible: Unknown

## 1.6 THE GENUS *PLEUROTUS*

The genus *Pleurotus* is a heterogeneous group of increasing economic value. Several species are of nutritional and/or medicinal importance (Gunde-Cimerman 1999,Guzman 2000, Cohen *et al.* 2002). Some *Pleurotus* species have the ability to absorb microelements from the different cultivation media, and thus they may present an excellent dietary source (Stajić *et al.* 2002).

Due to *Pleurotus*' ability to efficiently degrade natural lignin by excreting four ligninolytic enzymes laccase (Lac), manganese-dependent peroxidase (MnP), versatile peroxidase (VP), and aryl-alcohol oxidase (AAO)] (Muñoz *et al.* 1997), some species of the genus *Pleurotus* are commercially cultivated on different raw lignocellulosic materials (sawdust, paper, agricultural wastes, and other potential environmental pollutants) produced in enormous amounts worldwide.

However, the systematic position of some *Pleurotus* taxa was not well determined for a long time. Some taxonomic groups classified previously by morphological criteria were later shown, by the aid of molecular methods, to include several taxonomic entities (Brasier 1997).

Recently, several new taxonomic achievements in the genus *Pleurotus* were made by adding both mating and molecular studies. According to morphological characters, the subgenus *Coremiopleurotus* includes six morphological taxa (*P. cystidiosus, P. abalonus, P. smithii, P. fuscosquamulosus, P. gemmellari,* and *P. australis*). After testing their mating compatibility, three intersterility groups were revealed [*P. australis, P.cystidiosus* sensu lato, and *P. smithii* (Zervakis 1998)]. However, according to analysisof internal transcribed spacer 2 regions (ITS) sequences of ribosomal DNA (rDNA) (Zervakis et al. 2004), five phylogenetic species were presented within the subgenus *Coremiopleurotus* (*P. australis, P. abalonus, P. fuscosquamulosus, P. smithii,* and *P.cystidiosus*).

Previously defined mating groups were found to be either paraphyletic (Zervakis & Balis 1996, Brasier 1997) or to contain several distinct molecular lineages (Vilgalys & Sun 1994, Isikhuemhen et al. 2000). However, for a long time, it was unclear, whether *P. salignus* and *P. ostreatus* were different species or if they should have been regarded as synonymous.[13]

**Other edible wild species**

Many wild species are consumed around the world. The species which can be identified "in the field" (without use of special chemistry or a microscope) and therefore safely eaten vary widely from country to country, even from region to region. This list is a sampling of lesser-known species that are reportedly edible.

.

## 1.7 SCIENTIFIC NAME OF SOME SPECIES OF *PLEUROTUS*

| Scientific name of species | Origin |
|---|---|
| *P. ostreatus* (Jacq.) P. Kumm | Ukraine, Israel, Hungary, England, Yugoslavia, Hawaii,Bansladesh |
| *P. citrinopileatus* Singer | England |
| *P. cystidiosus* O.K. Miller | USA |
| *P. djamor* (Rumph. ex Fr.) Boedijn | Hawaii, Mexico |
| *P. eryngii* (DC.) Quél. var. *eryngii* | Israel, Hawaii |
| *P. eryngii* var. *tingitanus* Lewinsohn | Israel |
| *P P. cornucopiae* (Paulet) Rolland. | Belgium, Russia |
| *P. florida* Eger, nom. nud. | England |
| *P. pulmonarius* (Fr.) | Hawaii, Russia |

| | |
|---|---|
| Quél. | |
| *P. salignus* (Pers.) P. Kumm. | Israel |
| *P. salmoneo-stramineus* Lj. N. Vassiljeva | Belgium, Germany |
| P. smithii *Guzmán* | Israel, Haifa |

## 1.8 HISTORY OF MUSHROOM USE

There is evidence of mushroom use 13,000 years ago in Chile (Rojas and Mansur, 1995), although China has the first reliable evidence of wild fungi consumption, several hundred years BC (Aaronson, 2000). The Chinese value mushrooms for medicinal properties as well as for food. Ancient Romans and Greeks used mushrooms, particularly the wealthier classes (Buller, 1914).

The Pharaohs of Egypt enjoyed mushrooms so much that they decreed mushrooms could only be eaten by royal family and that no commoner could even touch them, thus giving the royal family the entire available supply of mushroom. In some parts of Eurasia, especially in Russia and Nordic countries, mushrooms are an important part of the diet. Several mushrooms are especially tasty and many are rich in nutrients. Mushrooms are also easily preserved, and historically have provided additional nutrition during winter.

Many prehistoric and a few modern cultures around the world used psychedelic mushrooms for ritualistic purposes. Mushroom cultivation reached the United States in the late 1800s with imported spores from Mexico. Some species such as death cap are extremely poisonous and have been deliberately used as instruments of assassination.
Medicinal mushrooms have a long and rich history of use. More than 2,000 years ago, Dioscorides knew that *F. officinalis* fought "consumption;" the Ice Man had *F. fomentarius* and *P. betulinus* with him; and the healers even shamans of Paleolithic peoples knew and used mushrooms as powerful medicines to fight illnesses. Interestingly, some mushrooms and their components are targetspecific in their antibiotic properties, whereas others have broader effects. With an increasing number of bacteria developing resistance to commercial antibiotics, such as MSRA (methicillin-resistant *S. aureus*) and *Pseudomonas*, extracts and derivatives from mushrooms hold great promise for novel medicines in modern times. The hypothesis, increasingly substantiated, is that mushrooms, especially polypores, provide a protective immunological shield against a vriety of infectious diseases.

Two thousand years have passed since the first century Greek physician Dioscorides included the larch polypore (Fomitopsis officinalis (Villars:Fr.) Bond. & Singer, Polyporaceae; syn. Laricifomes officinalis (Villars:Fr.) Kotlaba & Pouzar) in his De Materia Medica published approximately 65 C.E. Known then as agaricum or agarikon, and later as the quinine conk, it was used as a treatment for "consumption," a disease now known as tuberculosis.

The pharmaceutical industry has been slow to explore mushrooms for antibiotic activity, in part because basidiomycetous fungi are slower growing in fermentation and less yielding compared to the mold fungi, 9 such as the well known Penicillium notatum, the fungus from which Alexander Fleming discovered penicillinin (1928)..

## 1.9 NUTRITIONAL VALUE OF MUSHROOM

Mushrooms are very nutritious as food because they contain all the essential amino acids and are an excellent source of vitamins. Mushrooms give maximum nutritional[14] benefit only upon cooking. Mushrooms are relatively high in protein, averaging about 20% of their dried mass. Further they contribute a wide range of essential amino acids. Low in fat (between 3 and 2%) and high in fiber, mushrooms also provide several groups of vitamins, particularly thiamine, riboflavin, niacin, biotin, ascorbic acid and Vitamin D. All cultivated mushrooms are low in calories and are sodium free, fat free and cholesterol free. The nutritional facts[15] are given in Table 1.1

**Table 1.1: Nutrition Facts**

| **Nutrition Facts**<br>Serving size 5 medium mushrooms (84 gm) | | | |
|---|---|---|---|
| Amount per Serving | | | |
| Calories 20 | | | Calories from Fat 0 |
| | | | %Daily Value |
| Total Fat 0g | | | 0% |
| Saturated Fat 0g | | | 0% |
| Cholesterol 0mg | | | 0% |
| Sodium 0mg | | | 0% |
| Total Carbohydrate 3g | | | 1% |
| Dietary Fibre 1g | | | 4% |
| Sugars 0g | | | |
| Protein 3g | | | |
| Vitamin A 0% | | | VitaminC 2% |
| Calcium 0% | | | Iron 2% |
| Percent daily values are based on a 2,000 calorie diet. Daily values may be higher or lower depending on calorie needs for individuals: | | | |
| | Calories: | 2,000 | 2,500 |
| Total fat | Less than | 65g | 80g |
| Sat Fat | Less than | 20g | 25g |
| Cholesterol | Less than | 300mg | 200mg |
| Sodium | Less than | 2400mg | 2400mg |
| Total Carbohydrate | | 300g | 375g |
| Dietary Fibre | | 25g | 30g |
| Calories per gram: | | | |
| Fat 9 | Carbohydrate 4 | | Protein 4 |

A 50-g portion provides 1.5g of dietary fibre and is a rich source of copper; a source of vitamin $B_2$, niacin, folate, and selenium; supplies 6kcal (25kJ)[16].

Tab:1.2 **The Nutritional Value for:** mushrooms

| Description | Quantity | Energy (calories) | Carbs (grams) | Protein (grams) | Cholesterol (milligrams) | Weight (grams) | Fat (grams) | Saturated Fat (grams) |
|---|---|---|---|---|---|---|---|---|
| canned, drained, w/salt | 1 cup | 35 | 8 | 3 | 0 | 156 | 0 | 0.1 |
| cooked, drained | 1 cup | 40 | 8 | 3 | 0 | 156 | 1 | 0.1 |
| raw | 1 cup | 20 | 3 | 1 | 0 | 70 | 0 | 0 |

Fungi[17]of *Pleurotus* genus present a high content of proteins and vitamins and low content of fat. The protein content (from 1.54% to 3.10%) of *Pleurotus* fresh fruiting bodies proved to be similar to, or even higher than, the values observed in various vegetables but lower than the protein contents of eggs, meat and cheese.

With the aim of extending knowledge on chemical and nutritional[18]characteristics of commercial mushrooms widely consumed in Italy, fresh and processed mushrooms (*Agaricus bisporus, Pleurotus ostreatus* and *Boletus* group) were analysed fresh or after cooking. Results show that botanical variety, processing and cooking are all effective determinants of mushroom proximate composition. Dried mushrooms (*Boletus* group) after cooking show the highest nutritional value, essentially due to insufficient rehydration. Dietary fibre, chitin and beta glucans, all functional constituents of mushrooms, are present in variable amounts. Chitin level ranges from 0.3 to 3.9 g/100 g, while beta glucans, which are negligible in *Agaricus*, range from 139 to 666 mg/100 g in *Pleurotus ostreatus* and *Boletus* group. On average, a serving (100 g) of mushrooms guarantees from 9 to 40% of the daily recommendation of dietary fibre.

**AMINO ACIDS**

Amino acids[19] in water-extracts and total amino acids in hydrolysates of three cultivated mushrooms, *Agaricus bisporus, Pleurotus ostreatus* and *Lentinus edodes* were analyzed by amino acid analyzer to know the compositional differences depending on species and portions (pileus and stipe). Eighteen amino acids were identified and quantified. The total nitrogen and protein nitrogen contents were in the range of 1.67-6.24% and 0.88-2.42% (dry basis), respectively. The considerable differences were often found among species and portions of mushrooms. All of them were found to be higher in the pileus part. The free amino acids contents in water-extracts were in the range of 10.04-37.85mg/g (dry weight) and the total amino acids contents in hydrolysates were in the range of 53.37-120.15mg/g (dry weight). Glutamic acid, serine, histidine, and alanine were dominant in the free amino acids pool and glutamic, aspartic acid, histidine, and alanine were in the total amino acids pool.

## 1.10 HUMAM USE

### Edible mushrooms

Human use of fungi for food preparation or preservation and other purposes is extensive and has a long history: yeasts are required for fermentation of beer, wine and bread, some other fungal species are used in the production of soy sauce and tempeh. Mushroom farming and mushroom gathering are large industries in many countries.

Edible mushrooms[20] are used extensively in cooking, in many cuisines (notably Chinese, European and Japanese). For its higher nutritional value, many species of mushrooms are high in fiber, and provide vitamins such as thiamine ($B_1$), riboflavin ($B_2$), niacin ($B_3$), biotin ($B_7$),cobalamins ($B_{12}$) and ascorbic acid (C), as well as minerals, including iron, selenium, potassium and phosphorus. Mushrooms have been gaining a higher profile for containing antioxidants Ergothioncine and Selenium.

Several studies[21,22] indicates that the mushroom is about 16.5% dry matter and of the dry matter 7.4% is crude fiber, 14.6% is crude protein and 4.48% is fat and oil. Protein levels compare to shiitake at 18%, *P. ostreatus* at up to 30%, wheat at 13% and milk at 25% (all based upon dry weight). Fat levels are comparable to other mushroom species. Total sugar content is about 18.6% with high concentrations of galactose and low concentrations of glucose and maltose. Levels of oxalic acid, which can reduce the food value were low, and the mushrooms also contained low levels of vitamin C.

Most mushrooms that are sold in supermarkets have been commercially grown on mushroom farms. The most popular of these, *Agaricus bisporus,* is safe for most people to eat because it is grown in controlled, sterilized environments, though some individuals do not tolerate it well. Several varieties of *A. bisporus* are grown commercially, including: whites, crimini and portabello. Other cultivated species now available at many grocers include shiitake, maitake or hen-of-the-woods, oyster and enoki. A list of edible mushroom are attached (Table-1.11)

**Toxic mushrooms**

Chemical properties of mushrooms[23] is quite important because many species produce secondary metabolites that render them toxic, mind-altering, or even bioluminescent. Toxicity likely plays a role in protecting the function of the basidiocarp: the mycelium has expended considerable energy and protoplasmic material to develop a structure to efficiently distribute its spores. One defense against consumption and premature destruction is the evolution of chemicals that render the mushroom inedible, either causing the consumer to vomit the meal or avoid consumption altogether.

**Psychoactive mushrooms**

Psilocybin mushrooms possess psychedelic properties. They are commonly known as "magic mushrooms" or "shrooms", and are available in smart shops in many parts of the world, though some countries have outlawed their sale. A number of other mushrooms are eaten for their psychoactive effects, such as fly agaric, which is used for shamanic purposes by tribes in northeast Siberia. They have also been used in the West to potentiate, or increase, religious experiences. Because of their psychoactive properties, some mushrooms have played a role in native medicine, where they have been used to affect mental and physical healing, and to facilitate visionary states.

**Medicinal mushrooms**

Many fungi[24] are producers of antibiotics, including β-lactam antibiotics such as penicillin and cephalosporin. Widespread use of these antibiotics for the treatment of bacterial diseases, such as tuberculosis, syphilis, leprosy, and many others began in the early 20th century and continues to play a major part in anti-bacterial chemotherapy. The study of the historical uses and sociological impact of fungi is known as ethnomycology.

Currently, many species of mushrooms and fungi utilized as folk medicines for thousands of years are under intense study by ethnobotanists and medical researchers. Maitake, shiitake, and reishi are prominent among those being researched for their potential anti-cancer, anti-viral, and/or immunity-enhancement properties. Psilocybin, originally an extract of certain psychedelic mushrooms, is being studied for its ability to help people suffering from mental disease, such as obsessive-compulsive disorder. Minute amounts have been reported to stop cluster and migraine headache.

**Other human uses**

Fungi are also used extensively to produce industrial chemicals like lactic acid, antibiotics and even to make stonewashed jeans.[25-27] Mushrooms can be used for dyeing wool and other natural fibers. The chromophores of mushrooms are organic compounds and produce strong and vivid colors, and all colors of the spectrum can be achieved with mushroom dyes. Before the invention of synthetic dyes the mushrooms were the primary sources on dyeing textiles. This technique has survived in Finland, and many Middle Ages re-enactors have revived the skill again.

## 1.11 EDIBLE MUSHROOM SPECIES

| Species | Common name(s) |
|---|---|
| 1. ***Agaricus bisporus*** (J.E. Lange) Imbach (1946) | Button Mushroom, Champignon, Cremini, Hara Take, Masshuruumu, Portabello, Seiyou Matsutake, Shanpinion, Shuangbaomogu, Swiss Brown, Tsukuritake, White Mushroom |
| 2. ***Agaricus bitorquis*** (Quélet) Saccardo (1887) | Button Mushroom, French Mushroom, Pavement Mushroom, Spring Agaricus, Street Mushroom, Town Mushroom |
| 3. ***Auricularia auricula-judae*** (Bull.: Fries) Wettst. | Black Fungus, Chuaner, Hakekakeka, Hakeke, Heimuer, Kikurage, Mo-er, Mu-er, Senji, Setsuji, Wood Ear, Yuner |
| 4. ***Auricularia polytricha*** (Montagne) Saccardo | Aragekikurage, Black Fungus, Ear Fungus, Jews Ear, Kirurage, Maomuer, Mo-er, Mokurage, Mu-er, Muk Ngo, Tree Ear, Wood Ear, Yung Ngo |
| 5. ***Boletus edulis*** Bull.: Fries (1821) ($W^1$) | Cep; Cèpe de Bordeaux, Dajiao Gu (Fat Feet Mushroom), King Bolete, Meiwei Niuganjun, Penny Bun, Porcino; Steinpilz, Stone Mushroom, Yamadori Take, Zhutui Mo (Pig Leg Mushroom) |
| 6. ***Cantharellus cibarius*** Fries (1821) (W) | Anzu Take, Chanterelle, Gallinacci, Girolle, Jiyoujun |
| 7. ***Craterellus cornucopioides*** (Linnaeus: Fries) Persoon (1825) (W) | Horn of Plenty, Labajun, Trompeete des Morts |
| 8. ***Flammulina velutipes*** (Curtis: Fries) Singer (1951): | Enoki, Enoki Dake, Enoki Take, Enokitake, Furry Foot, Golden Mushroom, Golden Needle Mushroom, Japanese Golden Mushroom, Jinzhengu, Name Susuki, Nametake, Velvet Foot, Velvet Shank, Winter Mushroom, Yuki- |

| | Motase |
|---|---|
| **9.** ***Ganoderma lucidum*** (Curtis: Fries) P. Karsten | Ling Chi, Ling Chih, Ling Zhi, Mannentake, Reishi, Saiwai-Take, Sarunouchitake, The Panacea Polypore, Varnished Coke |
| **10.** ***Grifola frondosa*** (Dicks.: Fries) S.F.Gray | Hen of the Woods, Huishuhua, Kumotake, Limuo, Maitake, Sitting-Hen Mushroom, The Dancing Butterfly Mushroom |
| **11.** ***Hypsizygus tessulatus*** (Bull.: Fries) Singer (1947?) | Buna-Shimeji, Hon-Shimeji, Shimeji, Tamo-Motashi, The Beech Mushroom, Yamabiko Hon-Shimeji |
| **12.** ***Hypsizygus ulmarius*** (Bulliard: Fries) Redh(1984) | Elm Oyster Mushroom, Nire Take, Shirotamagitake, Shiro Tamogi Take |
| **13.** ***Lactarius deliciosus*** (Linnaeus: Fries) S.F. Gray (1821) (W) | Aka hatsu take, Meiwui Rugu, Pine Mushroom, Saffron Milk Cap |
| **14.** ***Lentinula edodes*** (Berkeley) Pegler (1975) | Black Forest Mushroom, Black Mushroom, Donggu, Golden Oak Mushroom, Huagu, Oak Mushroom, Shiitake, Xiangxin, Xianggu |
| **15.** ***Lepista nuda*** (Bull.: Fries) Cooke (1871): | Blewit, Murasaki Shimeji, Wood Blewit, Zixiangmo |
| **16.** ***Lyophyllum decastes*** (Fries: Fries) Singer (1951) | Fried Chicken Mushroom, Hatakeshimeji, Heye Lizhesan |
| **17.** ***Lyophyllum shimeji*** (Kawam.) Hongo | Bianhei Lizhesan, Hon-Shimeji, Yuxun Lizhesan |
| **18.** ***Marasmius oreades*** (Bolton: Fries) Fries (1838) (W) | Fairy Ring Champignon, Fairy Ring Mushroom, Mousseron, Yingbing Pisan |
| **19.** ***Morchella angusticeps*** Peck (W) | Black Morel, Conic Morel, Early Morel, Ko Togari Amigasa Take, Morel, Peck's Morel |
| **20.** ***Morchella costata*** (Ventenat) Persoon (1822) (W) | Morel |

| | |
|---|---|
| **21.** ***Morchella esculenta*** Linnaeus ex St.-Amans (W) | Amigasa take, Amigasa dake, Brain Mushroom, Gray Morel, Morel, Sponge Mushroom, White Morel, Yellow Morel, Yangdujun |
| **22.** ***Pholiota nameko*** (T. Ito) S. Ito & Imai | Huagu, Nameko, Namerako, Slime Pholiota, Viscid Mushroom |
| **23.** ***Pleurotus citrinopileatus*** Singer | Golden Oyster Mushroom, Golden Top Oyster Mushroom, Il'mak, Nireohma, Yellow Oyster Mushroom, Yellow Pleurotus, Yuhuangmo |
| **24.** ***Pleurotus cornucopiae*** Paulet: Fries | Golden Oyster Mushroom, Hime Hira Take, Nireohma, Tamogi Take, Yellow Oyster Mushroom, Yellow Pleurotus, Yuhuangmo |
| **25.** ***Pleurotus cystidiosus*** O.K. Miller | The Abalone Mushroom, The Maple Oyster Mushroom, Miller's Oyster Mushroom, Nangceer |
| **26.** ***Pleurotus djamor*** (Fries) Boedijn *in* de Wit (1959) | Fenceer, Hongo de la Pulpa, Oreja Blanca, Oreja de Palo, Oreja de Patancán, Pink Oyster Mushroom, Tabang Ngungut, Takiiro Hiratake, The Flamingo Mushroom, The Salmon Oyster Mushroom, The Strawberry Oyster |
| **27.** ***Pleurotus eryngii*** (De Candolle: Fries) Quélet | Boletus Of The Steppes, Caoyuan Ceer, King Oyster |
| **28.** ***Pleurotus ostreatus*** (Jacquin: Fries) P. Kummer (1871) | Abalone; Baoyugu, Blue Oyster (*P. columbinus*), Hiratake, Oyster Mushroom, Oyster Shelf, Straw Mushroom, Tamogitake, Tree Oyster, White Oyster Mushroom, Pinggu |
| **29. Pleurotus pulmonarius** (Fries) Quélet (1872) | Dhingri, Fengwuigu, Grey Oyster Mushroom, Houbitake (P. sajor-caju), Phoenix (-tail) Mushroom, The Indian Oyster, Usuhiratake |
| **30. Suillus bovinus (Linnaeus:** Fries) Roussel (1796) (W) | Ami take, Jersey Cow Bolete, Runiugangjun, Shallow-Pored Boletus |
| **31. Suillus luteus (Linnaeus:** Fries) Roussel (1796) (W) | Australian Cep, Hehuan Runiuganjun, Pine Bolete, Slippery Jack, Sticky Bun |

| | |
|---|---|
| **32. Tremella foliacea (Persoon:** S.F. Gray) Persoon | Brown Witch's Butter, Chayiner, Shiro Kikurage Modoki |
| **33. Tremella fuciformis** Berkeley | Baimuer, Shirokikurage, Shiro Kikurage Zoku, Silver Ear, Snow Fungus, White Jelly Fungus, Yiner |
| **34. Tremella mesenteriea** Retzius ex Fries | Common Witch's Butter, Huangjiner, Witch's Butter, Yellow Brain Fungus, Yellow Jelly Fungus |
| **35. Tricholoma terreum** (Schaeff.: Fries) P. Kummer (1871) | Griset; Zonghui Koumo |
| **36. Tuber aestivum Vittadini** (W) | Ekte Troffel, Kesatryfelli, Lanyz Letní, Musta Multasieni, Red-Grained Black Truffle, Sommartryffel, Sommertrøffel, Sommer Trüffel, St Jean Truffle, Summer Black Truffle, Summer Truffle, Trufa de Verano, Trufa de Verão, Truffe Anglaise, Truffe d'Eté, Truffe Noire à Grain Rouge, Truffe de la Saint-Jean, Trufla Letnia, Túbera de Verão, Tryufel' Letnii, Xia kuai Jun, Zomertruffel |
| **37. Tuber magnatum Pico & Vittadini (W)** | Alaca Keme, Banât Awbar, Graü Trüffel, Gray Truffle, Hui Bai Kuai Jun, Italian White Truffle, Italian Truffle, Lauchtrüffel, Piedmontese Truffle, Piedmont White Truffle, Piemontesertrøffel, Piedmont Truffle, Pitemengte Baikuijun, Tartufo Bigio, Tartufo del Piemonte, Trufa Blanca, Trufa de Piamonte, Trufa Gris, Truffe Blanche d'Alba, Truffe Blonde, Truffe des Provençaux, Truffe Grise d'Italie, Truffe Grise du Piémont, Weisse Italienische Trüffel, White Truffle, WinterWhite Truffle, Witte Truffel, Yidali Baikuijun |
| **38. Tuber melanosporum** Vittadini (W) | Black Truffle, Black Perigord Truffle, Echte Trüffel, Französische Trüffel, French Black Truffle, French Truffle, Hei Bao Kuai Jun Peiruigaode Heikuaijun, Périgord Black Truffle, Perigordtrøffel, Perigordtruffel, |

| | |
|---|---|
| | Perigord Trüffel, Périgord Tryffel, Schwarze Trüffel, Tartufo Nero, Trufa de Invierno, Trufa de Périgord, Trufa Negra, Trufa Violeta, Truffe des Gourmets, Truffe du Périgord, Truffe Franche, Truffe Noire, Truffe Périgourdine, Truffe Violette, Truffe Vraie, Trufla Czarnozarodnikowa, Tryufel' Chernyi, Tryufel' Nastoyashchii, Winter Black Truffle |

## 1.12 MEDICINAL PROPERTIES OF *PLEUROTUS* SPECIES

Recent studies on various *Pleurotus* species have shown a number of therapeutic activities, such as antitumour, immunomodulatory, antigenotoxic, antioxidant, anti-inflammatory, hypocholesterolaemic, antihypertensive, antiplatelet-aggregating, antihyperglycaemic, antimicrobial and antiviral activities(Table-1.3)

**Table 1.3 Cross-Index of Mushrooms and Targeted Therapeutic Effects**

FUNGI PERFECTI'S

# MYCOMEDICINALS®

## CROSS-INDEX OF MUSHROOMS AND TARGETED THERAPEUTIC EFFECTS*

| | anti-bacterial | anti-candida | anti-inflammatory | anti-oxidant | anti-tumor | anti-viral | blood pressure | blood sugar | cardiovascular | cholesterol reducer | immune system | kidney tonic | liver tonic | lungs/respiratory | nerve tonic | sexual potentiator | stress reducer |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Agaricus brasiliensis* (Himematsutake) | | | | | • | • | | • | | • | • | | | | | | |
| *Cordyceps sinensis* (Cordyceps) | • | | | • | • | • | • | • | • | • | • | • | • | • | • | • | • |
| *Fomes fomentarius* (Ice Man Polypore) | • | | | | | • | | | | | | | | | | | |
| *Fomitopsis officinalis* (Agarikon) | • | | • | | | • | | | | | | | | | | | |
| *Ganoderma applanatum* (Artist Conk) | • | | • | | • | | | | | | | | | | • | | |
| *Ganoderma lucidum* (Reishi/Ling Chi) | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | | • |
| *Ganoderma oregonense* (Oregon Polypore) | • | | | | • | | | | • | | • | | | | • | • | |
| *Grifola frondosa* (Maitake/Hen of the Woods) | • | • | | | • | • | • | • | | | • | | | | • | • | • |
| *Hericium erinaceus* (Yamabushitake/Lion's Mane) | • | | • | | • | | | | | | | | | | | • | |
| *Inonotus obliquus* (Chaga) | • | | • | | • | • | | • | | | • | | • | | | | |
| *Lentinula edodes* (Shiitake) | • | • | | | • | • | • | • | | • | • | • | • | | | • | • |
| *Phellinus linteus* (Mesima) | • | | • | | | • | | | | | | | | | | | |
| *Piptoporus betulinus* (Birch Polypore) | • | | • | | | • | | | | | • | | | | | | |
| *Pleurotus ostreatus* (Hiratake/Pearl Oyster) | • | | • | | | • | • | | • | • | • | | | | • | | |
| *Polyporus sulphureus* (Chicken of the Woods) | • | | | | | | | | | | | | | | | | |
| *Polyporus umbellatus* (Zhu Ling) | • | | • | | • | • | | | | | • | | | • | • | | |
| *Schizophyllum commune* (Suehirotake/Split-Gill) | | • | | | • | • | | | | | | | | | | | |
| *Trametes versicolor* (Yun Zhi/Turkey Tail) | • | • | | • | • | • | | | | | • | • | • | | | | |

*These statements have not been evaluated by the Food and Drug Administration.

**Antimicrobial activity**

*Pleurotus* spp. contain substances that exert antibacterial and antifungal activities. Extracts and isolated compounds, presumably produced as a defence mechanism against other organisms. Table 1.4 [28-33] summarizes recently reported antimicrobial activities of *Pleurotus* spp.

Table 1.4. Reported antimicrobial activities of *Pleurotus* spp.

| Species | | Effective against |
|---|---|---|
| *P. ostreatus* | Crude extracts from fermentation broth | Gram-positive, Gram-negative bacteria and *Aspergillus niger* |
| | Hexane-dichloromethane extract containing *p*-anisaldehyde | *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Aspergillus niger* and *Fusarium oxysporum* |
| | Various extracts; two main unidentified compounds | *Bacillus* spp., *Escherichia coli*, *Vibrio cholerae* and *Salmonella typhi* |
| *P. eryngii* | Eryngin – an antifungal peptide<br>Eryngeolysin – a haemolysin | *Fusarium oxysporum* and *Mycosphaerella arachidicola*<br>*Bacillus* spp. |
| *P. sajor-caju* | 12 kDa ribonuclease | *Fusarium oxysporum*, *Mycosphaerella arachidicola*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* |

## ANTIMICROBIAL TESTING

Reports on antimicrobial testing are also available.Preliminary antimicrobial testing[34-44] of petroleum ether ext-ract of *P. ostreatus* by agar-well diffusion produced zones of growth inhibition of 3.0 – 7.8 ± (0.1 – 0.9) mm for the gram positive bacteria, 5.0 – 8.2 ± (0.1 – 0.7) mm for the gram negative bacteria and 8.1 – 10.8 ± (0.1 – 1.6) mm for the fungal isolates tested (Table 1.5).

**Table 1.5.** Preliminary antimicrobial testing of *Pleurotus ostreatus* extracts. Inhibition zone diameter (mm ± SD)*

| @Isolates | *Pleurotus ostreatus* | | Standard drugs# | 0.1% (v/v) Tween-20 |
|---|---|---|---|---|
| | PE | AE | | |
| **Gram - positive bacteria** | | | | |
| *Staphylococcus aureus* ATCC 25923 | 7.5 ± 0.1 | 7.6 ± 0.3 | 27.5 ± 0.7 | G |
| *Staphylococcus aureus* (8) | 7.2 ± 0.9 | 7.1 ± 0.7 | 27.7 ± 0.9 | G |
| *Staphylococcus aureus* (2) | 7.0 ± 0.8 | 7.0 ± 0.6 | 28.1 ± 0.4 | G |
| *Staphylococcus epidermidis* (4) | 7.1 ± 0.8 | 7.0 ± 0.7 | 29.1 ± 0.5 | G |
| *Bacillus subtilis* ATCC 6633 | 7.1 ± 0.1 | 7.2 ± 0.2 | 27.8 ± 0.3 | G |
| *Bacillus subtillis* (5) | 7.8 ± 0.2 | 7.6 ± 0.8 | 28.4 ± 0.6 | G |
| *Bacillus licheniformis* (10) | 7.7 ± 0.2 | 7.7 ± 0.4 | 27.8 ± 0.4 | G |
| †*Lactobacillus acidophilus* (2) | 3.0 ± 0.2 | 3.2 ± 0.1 | 28.9 ± 0.4 | G |
| **Gram –negative bacteria** | | | | |
| *Salmonella typhi* ATCC 13391 | 7.5 ± 0.1 | 7.2 ± 0.4 | 28.1 ± 0.1 | G |
| *Salmonella typhi* (5) | 7.2 ± 0.3 | 7.0 ± 0.1 | 28.4 ± 1.3 | G |
| *Escherichia coli* ATCC25922 | 7.0 ± 0.0 | 7.0 ± 0.0 | 29.5 ± 0.1 | G |
| *Escherichia coli* (10) | 8.2 ± 0.7 | 7.6 ± 0.8 | 28.5 ± 1.0 | G |
| *Proteus* sp.(3) | 6.9 ± 0.5 | 6.5 ± 0.6 | 27.8 ± 0.7 | G |
| *Klebsiella pneumoniae* (4) | 7.1 ± 0.2 | 7.0 ± 0.1 | 28.0 ± 0.1 | G |
| ††*Pseudomonas aeruginosa* (3) | 5.6 ± 0.4 | 5.2 ± 0.2 | 28.2 ± 0.6 | G |
| *Haemophillus influenzae* (4) | 5.5 ± 0.5 | 5.0 ± 0.7 | 27.7 ± 0.9 | G |
| **Fungi isolates** | | | | |
| *Candida albicans* ATCC 1880 | 8.1 ± 0.1 | 8.0 ± 0.3 | 28.4 ± 0.0 | G |
| *Candida* sp. (10) | 8.2 ± 0.3 | 8.3 ± 0.1 | 28.2 ± 0.7 | G |
| *Saccharomyces cerevisiae* (4) | 10.8 ± 1.2 | 10.5 ± 1.6 | 28.8 ± 0.6 | G |

Figures in parentheses represent the number of isolates tested; † All and two of the tested *L. acidophilus* strains were sensitive to ciprofloxacin and *P. ostreatus*, respectively. †† Three (3) of the ciprofloxacin sensitive *Pseudomonas aeruginosa* isolates (6 0f 6) were also sensitive to *P. ostreatus*. G = Bacterial growth around the in phosphate buffered 0.1% Tween-20 control wells; # = growth inhibition zones for ciprofloxacin (5µg per well) and fluconazole (5µg per well) used as standard drugs. *The diameters of zone of inhibition were expressed in millimeter (mm) as mean ± standard deviation. @Seventy-nine (89.8%) of the 88 isolates showed sensitivity to *P. ostreatus*. PE = Petroleum ether extract; AE = Acetone extract.

Among the gram-positive bacteria tested, local isolates of *L. acidophilus* (2 of 8) and *B. subtilis* (5 of 5) were observed to be the least [3.0-3.2 ± (0.1 - 0.2) mm] and most [7.6 - 7.8 ± (0.2-0.8) mm] sensitive strains in both organic extracts. In gram-negative bacteria, local isolates of *E. coli* and *H. influenzae* exhibited the highest [7.6 – 8.2 ± (0.7 –0.8) mm] and lowest [5.0 - 5.5 ± (0.5-0.7) mm] sus-ceptibilities, respectively. Furthermore, highest suscepti-bility [10.5 - 10.8 ± (1.2-1.6) mm] by *S. cerevisiae* strains (4 of 4) and lowest susceptibility [8.0-8.1 ± (0.1-0.3) mm] by *C. albicans* ATCC1880 were observed among the fungal isolates. On the whole, 79 (89.8%) of the 88 isola-tes tested showed sensitivity to acetone and petroleum ether extracts of *P. ostreatus* (Table 1.5).

Based on the results of this study, it was concluded *P. ostreatus*, an edible oyster mushroom has antioxidant potentials and possess a broad-spectrum antibacterial and antifungal activity, optimisable by multiple organic mycelia extraction.

**Antiviral activity**

Antiviral activity have been observed in *Pleurotus Spp*. Mushrooms contain substances that exert direct or indirect antiviral effects as a result of immunostimulatory activity[45]. Inhibitory activity against human immunodeficiency virus (HIV)-1 reverse transcriptase has recently been demonstrated for *P. sajor-caju* and *P. pulmonarius* hot water extracts[46] . Anti-HIV activity was also demonstrated for a ubiquitin-like protein isolated from *P. ostreatus* fruiting bodies . Moreover, Zhang *et al.* demonstrated that, in contrast to water-insoluble b-glucans isolated from *P. tuber-regium* sclerotia, their corresponding water-soluble sulphated derivatives exert antiviral activities against herpes simplex virus type 1 and type 2. The effect is presumably elicited by the binding of sulphated b-glucans to viral particles, thus preventing them from infecting the host cells.

**Cardiovascular disease protection and antihyperglycaemic activities**

Oyster mushrooms possess bioactive compounds with hypocholesterolaemic activities, such as polysaccharides, mevinolin and other statins[47] . It has recently been reported that *P. citrinopileatus* fruiting body extracts exerted antihyperlipidaemic effects. Serum triglyceride and total cholesterol levels were lowered in hyperlipidaemic rats supplemented with the extracts, while high-density lipoprotein levels were significantly increased[48]. Similar effects were noted when powdered *P. ostreatus* fruiting bodies or a water-soluble polysaccharide extracted from *P. citrinopileatus* fermentation broth were fed to hypercholesterolaemic or diabetic rats, respectively.

*Pleurotus* species also possess blood-pressure-lowering activity. Recently, *P. cornucopiae* has exhibited antihypertensive activity; this might be due in part to D-mannitol, which inhibits angiotensin I converting enzyme [49].

A methanol extract of *P. florida* fruiting bodies significantly inhibited platelet aggregation. The antiplatelet-aggregating activity, along with the anti-inflammatory activities discussed above, suggest its potential therapeutic use against vascular disorders, but the exact mechanism of these activities is unknown[50] .

Antihyperglycaemic activity was demonstrated with a water-soluble polysaccharide from *P. citrinopileatus* fermentation broth. The polysaccharide was effective in lowering blood glucose levels in diabetic rats.

**Antitumour activity**

*Pleurotus* spp. extracts and isolated compounds such as polysaccharides, proteins and other substances that possess antineoplastic activities *in vitro* and *in vivo*. Antitumour activities have been observed in various crude extracts of *Pleurotus* Species . Methanol extracts of *P. florida* and *P. pulmonarius* fruiting bodies significantly reduced solid tumours in mice[51-52]. *P. ostreatus* mycelium extract, alone and combined with the chemotherapeutic agent cyclophosphamide, inhibited *in vivo* tumour growth in mice. The combined administration of the extract with cyclophosphamide decreased the degree of leukopenia compared to administration of cyclophosphamide alone[53]. A water extract of *P. ostreatus* exhibited the most significant cytotoxicity by inducing apoptosis of human carcinoma cells, when compared to many other mushroom extracts. It has been suggested that the active compounds in the extract were water-soluble proteins or polypeptides[54] . Among the components of such extracts, polysaccharides are well-documented as potent antitumour and immunomodulating substances[55-56]. Many polysaccharides from *Pleurotus* spp. have been isolated and identified[57-67] . For some of them, important medicinal properties, including antitumour activities, have been shown. *P. tuber-regium* polysaccharides, extracted from mycelium and fruiting bodies, effectively inhibited solid tumour proliferation in mice.

Antitumour effects have also been shown on different human tumour cell lines[68,69] . Wong *et al.* showed that *P. tuber-regium* polysaccharides exerted antitumour activity, through cytotoxicity and antiproliferative activity, against human leukaemia cells *in vitro*. The polysaccharides induced apoptosis and caused cell cycle arrest. Compared to native *P. tuber-regium* polysaccharides, their corresponding carboxymethylated or sulphated derivatives showed higher antitumour activity, presumably because of their higher water solubility and relatively extended flexible chains[70-73] . A novel a-glucan from *P. ostreatus* mycelium induced apoptosis of colon cancer cells *in vitro* and water-soluble polysaccharides extracted from *P. citrinopileatus* fermentation broth have been shown to reduce the number of metastatic tumour nodules in tumour-bearing mice .

Antitumour properties have also been demonstrated for *Pleurotus* spp. proteins, proteoglycans, and DNA. A lectin isolated from *P. ostreatus* potently inhibited growth of sarcoma and hepatoma in mice and prolonged their lifespan[74] . A *P. eous* lectin exerted antiproliferative effects on human tumour cell lines while showing no cytotoxicity[75] . Furthermore, two ribonucleases isolated from *P. sajor-caju* and *P. ostreatus* fruiting bodies exhibited antiproliferative effects on tumour and leukaemia cell lines[76,77] . Another protein, eryngeolysin, isolated from *P. eryngii* fruiting bodies, exhibited cytotoxicity against leukaemia cells[78] . Water-soluble proteoglycans were purified from *P. ostreatus* mycelium and exerted antitumour activity in sarcoma-bearing mice. Proteoglycans injected into mice reduced the number of tumour cells by cell cycle arrest [79]. Moreover, DNA isolated from *P. ostreatus* fruiting bodies administered to mice with solid Ehrlich carcinoma significantly increased the lifespan of mice[80].

**Immunomodulatory and antimitogenic activities**

Several compounds from *Pleurotus* species with immunostimulatory activities on humoral and cell-mediated immunity have been isolated. Water-soluble polysaccharides extracted from *P. citrinopileatus* fermentation broth administered to mice resulted in a significant increase in the number of macrophages, T, CD4+ and CD8+ cells[81]. Glucans isolated from *P. florida* fruiting bodies activated the phagocytic response of mouse

macrophages *in vitro* and significantly induced the proliferative response as well as phagocytic activity of fish leukocytes *in vitro*. Moreover, proteoglycans from *P. ostreatus* mycelia exerted immunomodulatory effects by elevating mouse natural killer cell cytotoxicity and by macrophage stimulation. DNA isolated from *P. ostreatus* fruiting bodies stimulated mouse natural killer cytotoxic activity *in vitro* .

Antimitogenic effects of *Pleurotus* spp.-derived compounds on immune cells have also been reported. A ribonuclease isolated from *P. sajor-caju* fruiting bodies exerted antiproliferative effect on murine splenocytes , while eryngeolysin from *P. eryngii* inhibited the stimulated mitogenic response of murine splenocytes. Furthermore, *P. flabellatus* lectin did not exhibit any mitogenic activity towards mouse T cells [82].

**Antioxidant and gene protective activities**

Antioxidant compounds prevent oxidative damage related to aging and diseases, such as atherosclerosis, diabetes, cancer and cirrhosis. Mushrooms that contain antioxidants or increase antioxidant enzyme activity may be used to reduce oxidative damage in humans[83] .

Of 89 mushroom species tested, an extract from *P. cornucopiae* possessed the most effective antigenotoxic and bio-antimutagenic activities when tested on *Salmonella typhimurium* and *Escherichia coli*. Furthermore, *P. cornucopiae* extracts significantly reduced $H_2O_2$-induced DNA damage in Chinese hamster lung cells and *P. ostreatus* extract mitigated genotoxicity, as shown by the fact that it suppressed DNA damage induced by various mutagens in the Drosophila DNA repair test . On the other hand, a water extract of *P. sajor-caju* fruiting bodies had no genoprotective effects since it did not prevent $H_2O_2$-induced oxidative damage to cellular DNA[84].

Methanol extracts of *P. ostreatus* and *P. cystidiosus* fruiting bodies possessed antioxidant, reducing power, radical scavenging and iron chelating activities that were higher than those of other commercial mushrooms. On the other hand, Elmastas *et al.* and Dubost *et al.* reported that oyster mushroom extracts possessed only moderate antioxidant

activities compared to other edible mushrooms. The antioxidant activity was positively correlated with total polyphenol content. Furthermore, Lee *et al.*[85] showed that *P. citrinopileatus* extracts prepared from fruiting bodies were more effective than those from mycelium and fermentation broth filtrate presumably due to a higher amount of total phenols. Methanol extracts of *P. florida* and *P. pulmonarius* fruiting bodies showed similar antioxidant activities, and an ethanol extract from *P. citrinopileatus* fruiting bodies had antioxidant activities comparable to those from *P. eryngii*, *P. ferulae* and *P. ostreatus* mushrooms[85,86] .

*P. citrinopileatus* fruiting body extracts have shown antioxidant activities *in vitro* and in hyperlipidaemic hamster rats. Extracts added to a high-fat diet increased the activities of antioxidant enzymes in rats . *P. ostreatus* mushroom extracts had antioxidant properties in aged and $CCl_4$-induced liver damaged rats, as indicated by significant increases in concentrations of antioxidants and antioxidant enzymes . Pleuran, a b-glucan isolated from *P. ostreatus*, had a positive effect on the antioxidant status of rats and decreased precancerous lesions induced in rat colon [87]. A polysaccharide-peptide complex isolated from *P. abalonus* fruiting bodies prolonged the lifespan of senescence-accelerated mice. Gene expression of antioxidant enzymes was up-regulated and consequently their activities were increased[88] .

**Anti-inflammatory activity**

Jose *et al.*showed that methanol extracts of *P. pulmonarius* and *P. florida* fruiting bodies decreased induced paw oedema in mice and ameliorated acute and chronic inflammation, respectively. Pleuran has also been shown to possess anti-inflammatory activity by exerting antioxidant and immunomodulatory effects on rats with induced colitis .Hypersensitive immune responses, such as inflammation in delayed allergy, were suppressed by an ethanol extract of *P. eryngii*. It exhibited antiallergic activity after oral or percutaneous administration to mice with oxazolone-induced type IV allergy[89].

## 1.13 PHYTOCHEMICAL CONSTUENTS

### Chemical Composition

In general, the gross composition of mushrooms is water , protein , fat , carbohydrates , fiber and ash (ash is mainly composed of salts, metals and so forth). Active metabolites can be isolated from fruiting bodies, pure culture *mycelia* and culture filtrate, and now-a-days many attempts are being made to obtain active metabolites from *mycelia* through submerged fermentation culture to obtain cheaper preparations. Kawagishi was the first to separate an active anticancer compounds purified from the sodium hydroxide extract of the fruit body of ABM(Agaricus Blazei Mushroom). The author detected polysaccharides with apparent antitumor activity and comprised a protein complex composed of 43.4% protein and 50.2% carbohydrates[90] . ABM fruiting bodies in different stages of maturity contain ∝-glucans and ß-glucans: the yield and structural diversity of glucans increase as the fruiting bodies mature; so the time of the harvest and conservation is of great importance, to obtain the best extract.

### The ∝and ß-glucan Structure

ABM glucans are side branches of a (1-6)-ß-backbone as found by Dong and Ohno, who described that active fraction of ß-glucans of ABM fruiting bodies had a (1-6)-ß-backbone structure (or functional center) with (1-3)-ß-side branches in the ratio of 1 : 2 [91]; while the linear (1,6)-ß-glucan seems to be inactive [92] (Fig-1.4). The biochemical importance of (1-3)-ß-side branches has been confirmed and has shown the enhancement of the immunomodulatory activity of polysaccharides [93]; and Mizuno reported an important antitumor activity linked to the water-soluble (1-6)-(1-3)-ß-D-glucan. However, a significant increase of water-soluble (1-4)-∝-glucan with apparent antitumor activity occurs during maturation [94]; so probably cap-opened, more fragile mature fruiting bodies of ABM should be selected over immature ones for the production of nutraceuticals because they contain the most useful glucans [95]. In addition, an ∝-1,6 and ∝-1,4 glucan

complex [96] and a glucomannan with a main chain of ß-1,2-linked D-mannopyranosyl residues have been isolated from this mushroom and found to inhibit tumorigenesis[97].

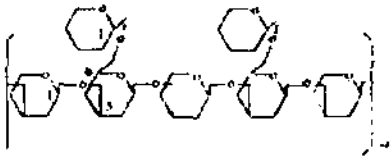


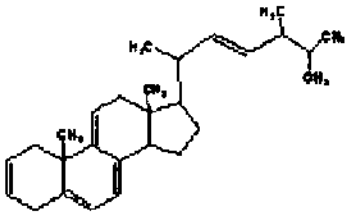
**Figure 1.4.** (1–6)-ß-backbone structure (or functional center) with (1–3)-ß-side branches.

**Mechanisms of Tumorigenesis and Carcinogens**

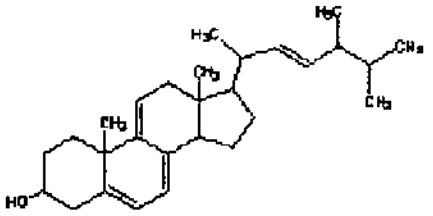
These results suggest that whole-mushroom extracts contain compounds that may modulate tumorigenesis and carcinogenesis at different stages and/or may act at the same stage through different mechanisms. Responses to such highly different polysaccharides are likely to be mediated by different cell-surface receptors, which may be present only on specific subsets of cells, and may trigger distinct downstream responses. A combination of such responses involving different cell subsets could conceivably provide greater tumor inhibition than could be induced by a single polysaccharide. Nevertheless, a very important problem is the wide number of different and only partially homogeneous ABM extracts used to study the pharmacological activities of its constituents representing a difficult challenge to establish the best extract and active substances. Thus all these similar constituents could potentially provide additive, or even synergistic, effects in the prevention and treatment of cancer. Moreover they could interfere with other substances or healthy physiological functions. This has been shown by an *in vitro* study in which increasing fractionations of an ABM extract enhanced some biological activities but abolished others [98,99].

## 1.14 PHYTOCHEMICAL INVESTIGATION OF *PLEUROTUS* GENUS

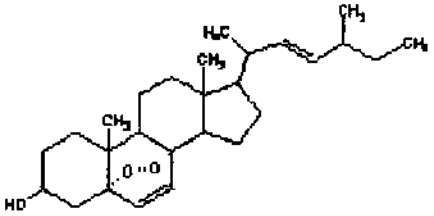
Mushrooms belonging to the *Pleurotus* genus[100], more known as oyster mushrooms, alluding to its appearance, have brought great interest due to the versatility of their crop, which contains a wide substrate variety, including agroindustrial waste. However, studies, made around, its chemical composition have been left aside and are focused in evaluating the optimization of processes involving biomass production. *Sajor-cajú* species is an edible mushroom of clear colors, saprophyte, primary degrader and wood decomposer from which reports have not been found besides the isolation and quantification of ergosterol [IV]. Here showed information related to triterpenic composition and the fatty acids identified, and as in Trigos A, found ergosterol IV accompanied by nine additional triterpenes identified as Ergosta-2,5,7,9(ll),22-pentaene I, Ergosta-5,7,9(ll),22-tetraen-ß-ol II, Ergosta-5α ,8α epidioxy-6,22-dien-ß-ol III, Ergosta-5,7,22-trien-ß-ol (Ergosterol) IV, Ergosta-7,22-dien-ß-ol V, 19-Norergosta-5,7,9,22,tetraen-ß-ol VI, Ergosta-7-en-ß-ol VII, Ergosta-3ß-5α,6ß-trihydroxy-7,22-diene VIII, Ergosta-4,6,8(14),22-tetraen-3-one IX and Ergosta-4,6,15(16),22-tetraen-3-one X.



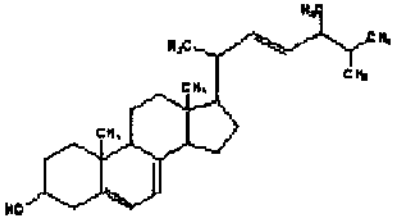
I



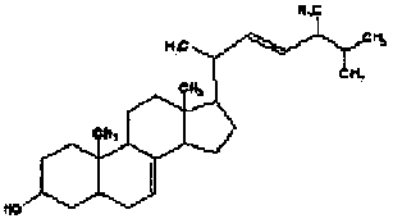
II



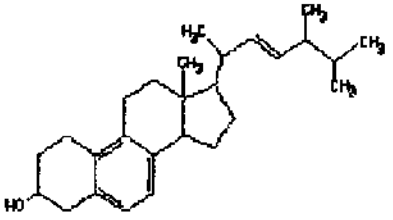
III



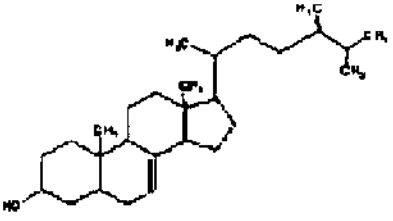
IV



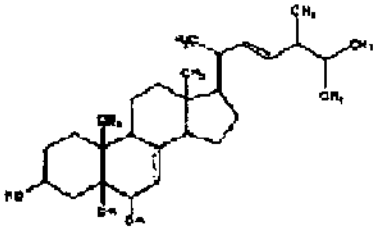
V



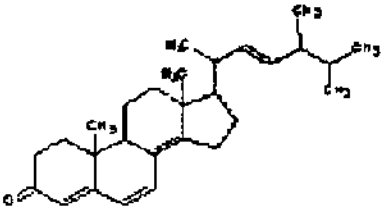
VI



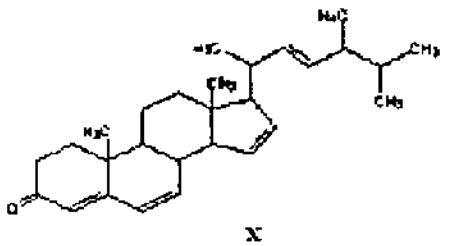
VII



VIII



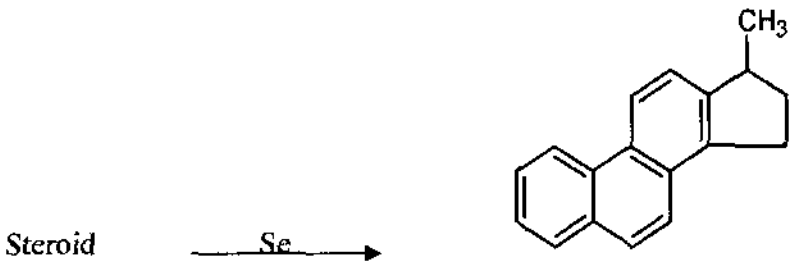
IX



X

## 1.15 STEROIDS

Compounds which produce Diel's hydrocarbon on selenium dehydrogenation (3-methyl-1,2-cyclopenteno-phenanthrene) are known as steroids [101]. Most eukaryotic cells contain and synthesize sterols. Whereas cholesterol is the unique sterol of vertebrates and ergosterol the major sterol of most fungi and some unicellular algae, most higher plants contain a complex mixture in which cholesterol is a minor component and 24-ethyl sterols (sitosterol and stigmasterol) account for more than 60% and 24-methyl sterols account for less than 40%.



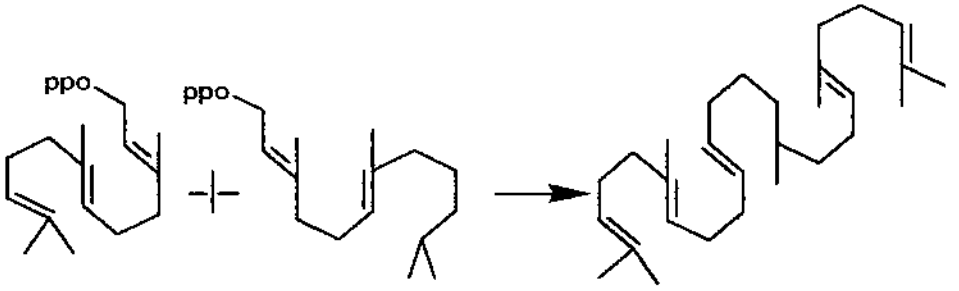

Diel's hydrocarbon

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

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

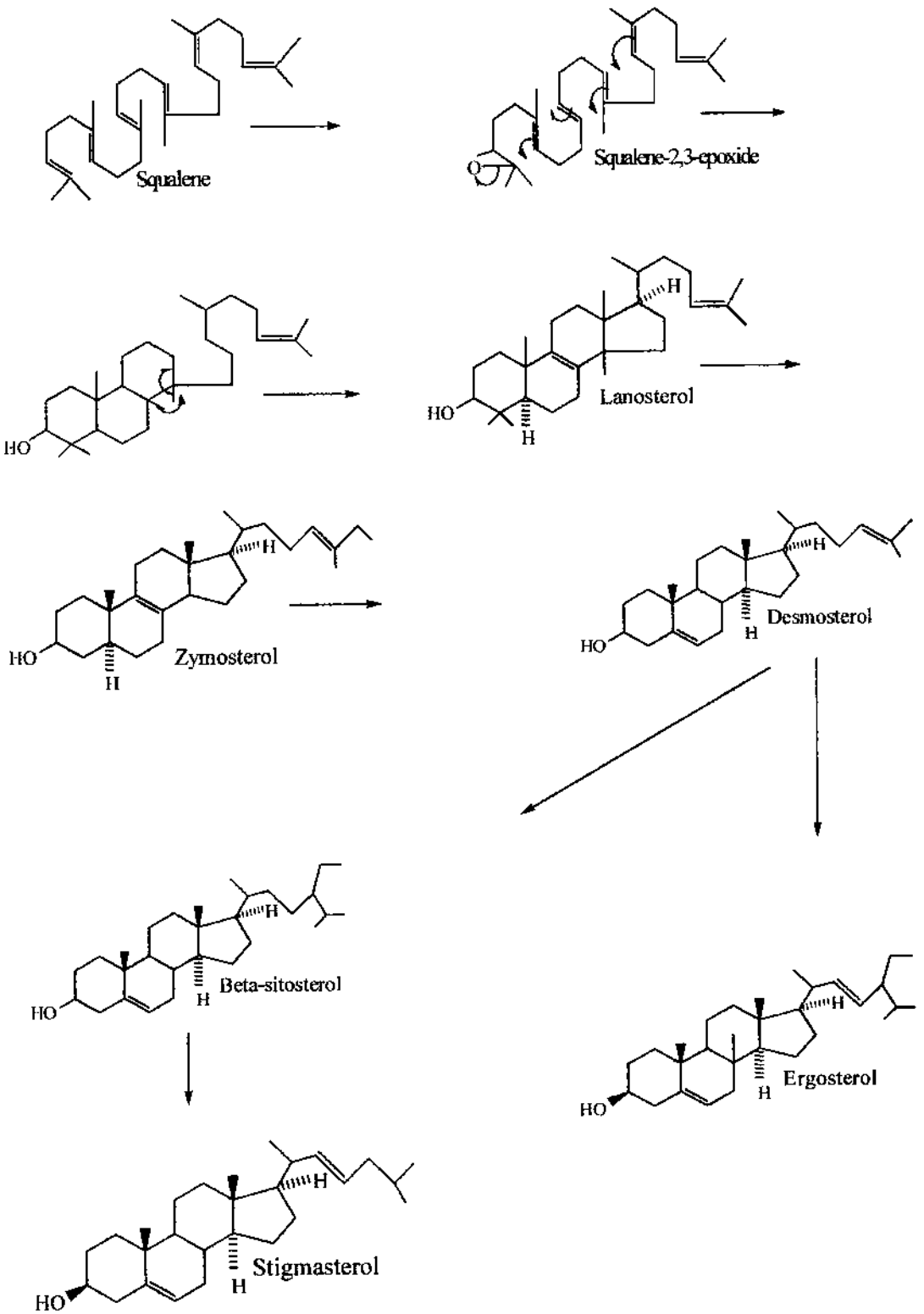
## 1.16 BIOSYNTHESIS OF STEROIDS

Steroids [102,103](Agarwal, 1994) are not triterpenes as they possess $C_{27}$-$C_{29}$ skeletons; rather, althouth both of them are derived from the same $C_{30}$ precursor squalene. This squalene is derived from two farnesyl-pyrophosphate units, upon joining each other in the unusual 'head to head' fashion.




Squalene

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




**Scheme 1.1: Biosynthesis of phytosterols.**

# 2. METHODOLOGY

# 2. METHODOLOGY

## 2.1 GENERAL METHODS

The chemical investigation of a mushroom can be divided roughly into the following major steps :

a) Collection and proper identification of the mushroom material

b) Preparation of mushroom sample

c) Extraction with suitable solvents

d) Fractionation of the extracts and isolation of compounds

e) Structural characterization of the fractions and the purified compounds

## 2.2 DISTILATION OF SOLVENTS

Analytical grade solvents and chemicals used in the experiments. All solvents and reagent used in the experiments were purchased from E. Merk (Germany), BDH (England). The analytical grade solvents (n-hexane, dichloromethane methanol) were used. Solvents other than analytical grade were distilled before use.

## 2.3 EVAPORATION

For the removal of solvents and for concentration all evaporations were carried out under reduced pressure using rotary vacuum evaporator at bath temperature not exceeding 40°C.

## 2.4 DETECTION

### 2.4.1 UV-LIGHT

The fluorescent compounds on the thin layer chromatogram plates were observed under UV- light at 254 and 350 mm. Some of the compounds appeared as fluorescing spots while the others are dark spots under the UV-light.

The developed chromatogram is viewed visually to detect the presence of colored compounds.

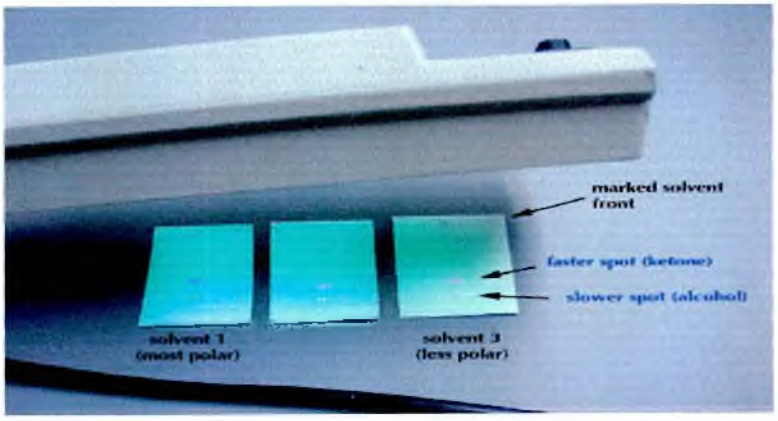



**Fig 2.1: Visualization/detection of compounds in UV lamp**

### 2.4.2 IODINE CHAMBER

Iodine vapour has also used as a general reagent to detect spots in the TLC plates. A closed jar or tank with powdered iodine was used to identify the spots. The compounds that appeared as brown spots are marked. Unsaturated compounds absorb iodine. Bound iodine is removed from the plate by air blowing.

## 2.5 PREPARATION OF THE REAGENTS

### 2.5.1 VANILLIN-SULPHURIC ACID REAGENT

Concentrated sulphuric acid (400 ml) and absolute alcohol (100 ml) were mixed in a beaker (kept in an ice bath). Vanillin (0.25 g) was added to the mixture of alcohol and sulfuric acid, cooled and named as vanillin-sulphuric acid reagent and used for spraying the TLC plates.

In TLC plates the spots responding to vanillin-sulphuric acid indicates the presence of terpenoids.

Fig 2.2: Vanillin-sulphuric acid spray

## 2.6 CHROMATOGRAPHIC TECHNIQUES

Thin layer chromatography (TLC) and vacuum liquid chromatography (VLC), column chromatography and gas liquid chromatographic techniques used during the course of the study.

### 2.6.1 THIN LAYER CHROMATOGRAPHY (TLC)[104]

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

1. Precoated TLC plates: 0.2mm thin coatings of silica gel on glass plates or aluminium sheets were used.
2. Manually prepared silica gel coated glass plates were used.

### 2.6.2 PREPARATION OF PLATES

Thin layer chromatographic plates were prepared by spreading a film of an aqueous slurry (gel: water = 1:2 w/v) of silica gel G-60 PF254 (E of merck7731) over the entire surface of the glass plates (6cm x 12 cm) by means of spreader. This thickness of the silica gel layer was 0.2 mm. The plates were dried the air and finally activated by heating at $110^0$C for 1 hour followed by cooling at room temperature.

### 2.6.3 SAMPLE APPLICATION (SPOTTING THE PLATES)

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

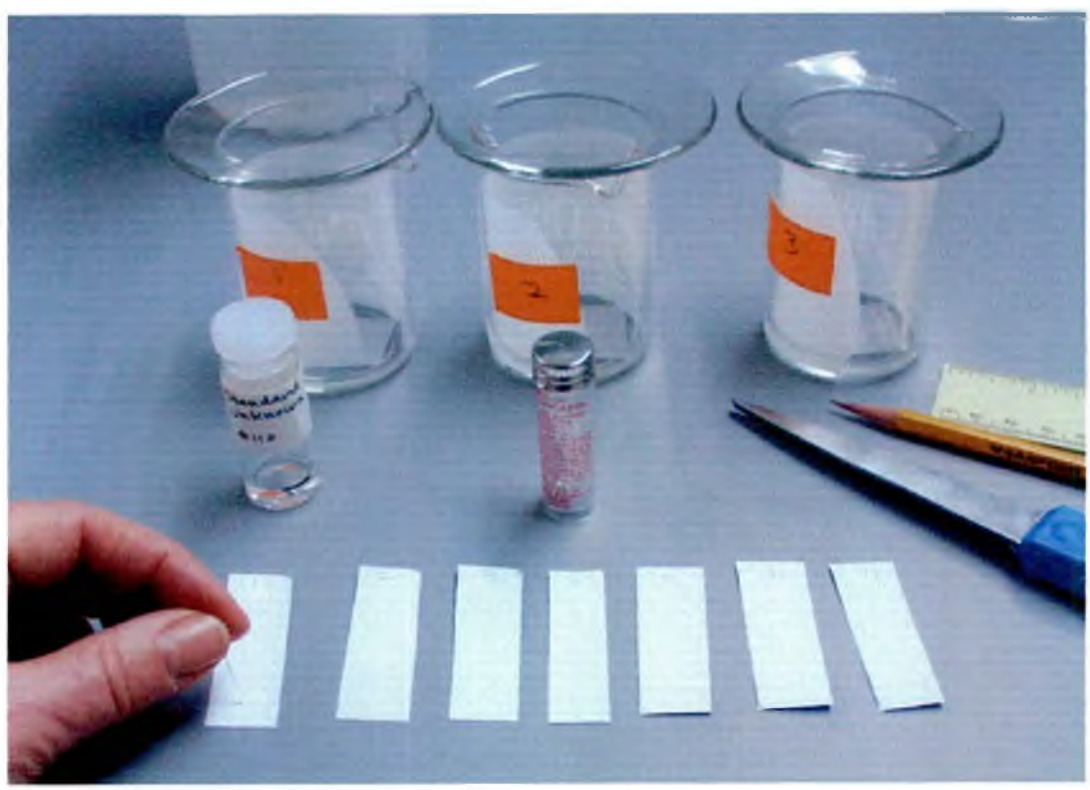


**Fig 2.3: process of spotting**

### 2.6.4 SOLVENT SYSTEMS

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

(i) n- Hexane
(ii) Dichloromethane
(iii) Methanol
(iv) Hexane: Dichloromethane (in different ratio to increase polarity)
(v) Hexane:Methanol (in different ratio to increase polarity)
(vi) Dichloromethane: Methanol (in different ratio to increase polarity)

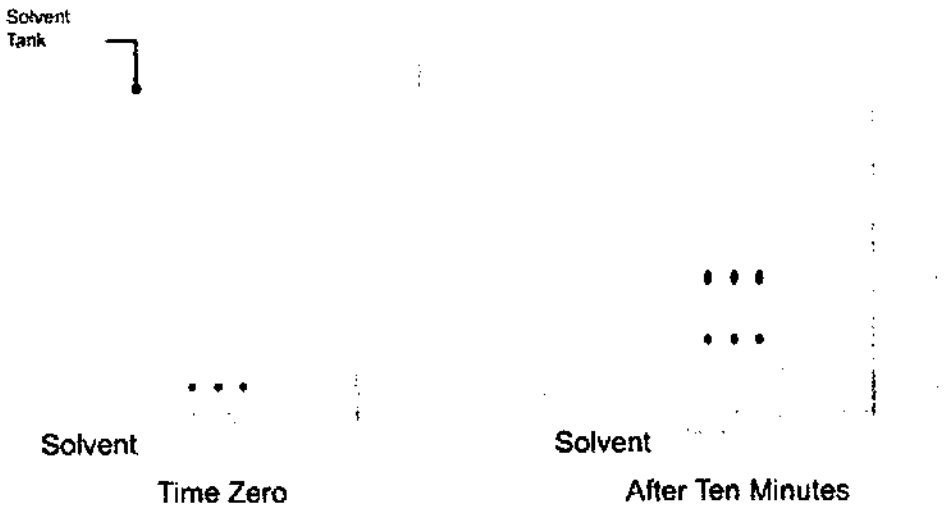



Fig 2.4: Elution of TLC plate

### 2.6.5 PREPARATION OF TLC TANK

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

### 2.6.6 DETECTION OF SPOTS

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

1. Examination under UV lights in different wavelength, 254 and 350 nm.
2. The plates were exposed to iodine vapor for several minutes.
3. The plates were sprayed with vanillin-sulfuric acid regent (1.0%) followed by heating in an oven at $120^0$C for 15 minutes.

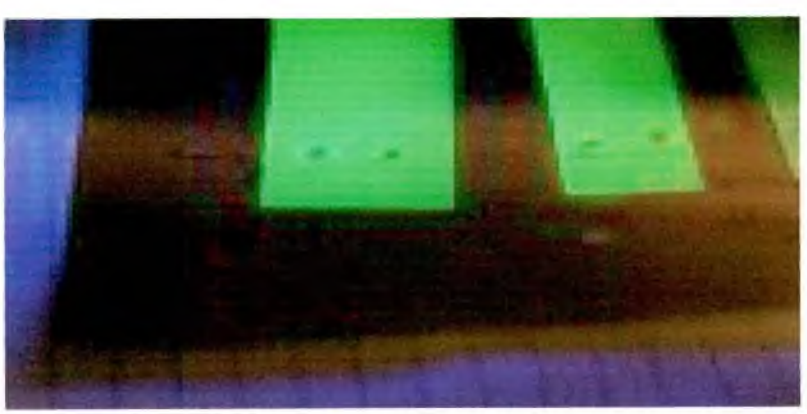


Fig 2.5:Irrigated TLC plate under UV lamp

### 2.6.7 THE $R_f$ VALUE

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

$$R_f = \frac{\text{Distance from sample front}}{\text{Distance from solvent front}}$$

Usually, the $R_f$ value is constant for any given compound under a set condition of temperature, solvent, plate thickness etc. and it corresponds to a physical property of that compound[105].

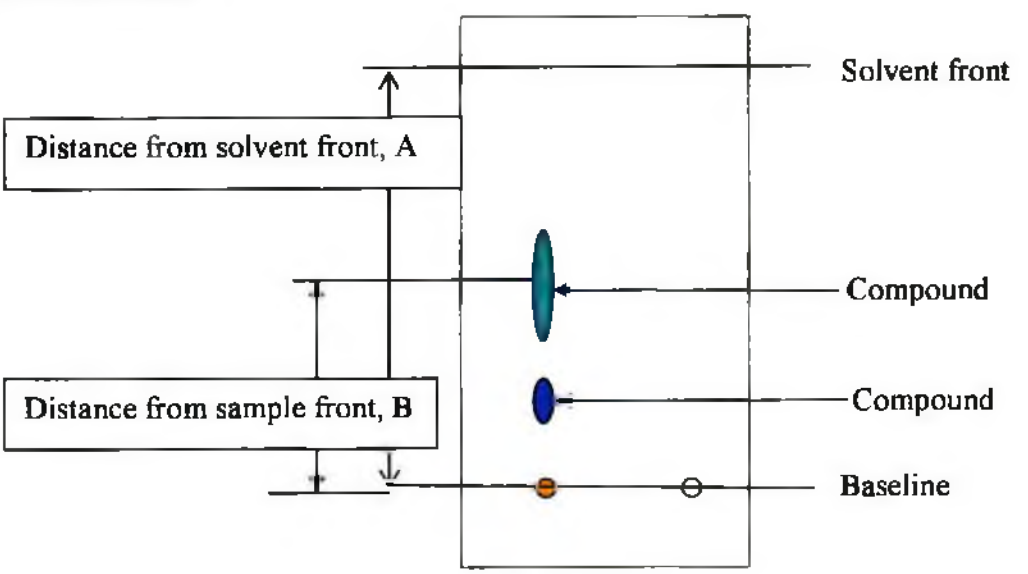



***Fig 2.6: A Plate for the calculation of $R_f$ value***

## 2.7 COLUMN CHROMATOGRAPHY

For normal phase column chromatography, silica gel of particle size 230-400 mesh from (Merck) was used and separation was performed by gravitational flow with solvents of increasing polarity. The sample was applied into the column either as a solution or in a powdered form. The eluted samples were collected in several test tube and were monitored by TLC to make different fractions on the basis of $R_f$ values.

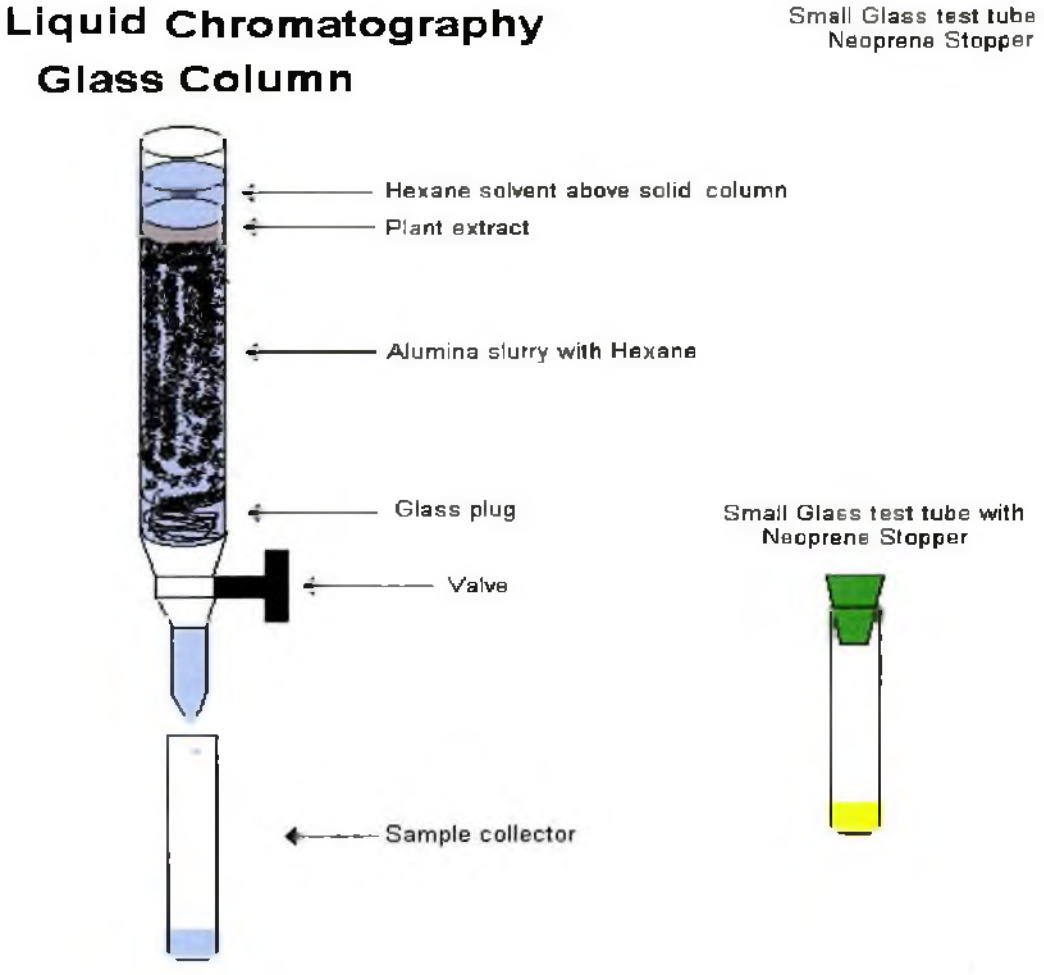



**Fig 2.7: Various part of a column**

## 2.7.1 PROCEDURE FOR MICRO SCALE FLASH COLUMN CHROMATOGRAPHY

In micro scale flash chromatography, the column does not need either a pinch clamp or a stopcock at the bottom of the column to control the flow, nor does it need air-pressure connections at the top of the column. Instead, the solvent flows very slowly through the column by gravity until we apply air pressure at the top of the column with an ordinary Pasteur pipet bulb.

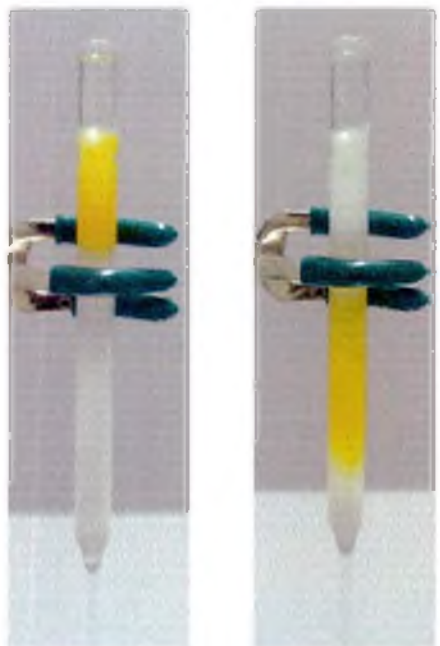


**Fig 2.8: Various stages in micro scale column**

## 2.7.2 PREPARATION OF COLUMN (FOR MICRO SCALE OPERATION)

A Pasteur pipet was plugged with a small amount of cotton to prevent the adsorbent from leaking. The Pasteur pipet was filled with the slurry of column grade silica gel with a stream of solvent using a dropper. It was ensured that the "Sub Column" is free from air bubbles by recycling the solvents several times. The samples were applied at the top of the column. Elution was started with petroleum ether followed by increasing polarity.

## 2.8 GAS CHROMATOGRAPHY (GC)

Chromatography[106] is a physical process of separation in which the components to be separated are distributed between two phases a stationary phase having large surface area usually coated with a nonvolatile liquid stationary phase and a mobile phase (a gas or liquid) moving in contact with the stationary phase. Different forms of chromatography are named according to the physical state of mobile phase employed. Gas chromatography (GC) is fundamentally a separation technique but it provides identification of a compound and with due calibration permits quantitative estimation as well. Today it is used by analytical chemists in almost every branch of science as a powerful analytical tool. It finds application in various fields such as gases and pollutants, petroleum products, oils and fats, foods and flavors, drugs, beverages, and a number of varied materials.

### 2.8.1 THE PRINCIPLE ADVANTAGES OF GC

The principal advantages of GC to an analyst are

i. The technique has strong separation power and even quite complex mixtures can be resolved into constituents.
ii. It is a micro method and only a few mg samples are enough for analysis; sensitivity of the method is very high.
iii. Speed of analysis is quite fast.
iv. Gives good precision and accuracy.
v. It involves relatively simple instrumentation; operation of a gas chromatograph and related calculation do not require highly skilled personnel and thus the technique is very suitable for routine analysis.
vi. The cost of equipment is relatively low and life is generally long.

maintain the k-value; at the same time, out of the swept amount some portion will go into sorbent at the next point in the column, again to maintain the k-value. This goes on successfully and continuously and as a whole the band for each component move further in the column and having the shape of, more or less Gaussian Distribution (peak shape). Now components having varying affinities for the sorbent (stationary phase) will be held up in the latter to different degrees and consequently will be moved along the column with different speeds; if a column of sufficient length is chosen, these components will emerge out of the column at different intervals. This is how the separation of components is achieved in GLC.

## 2.9 CRYSTALIZATION

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

## 2.10 SPECTROSCOPIC TECHNIQUES

### 2.10.1 ULTRA-VIOLET SPECTROSCOPY (UV)

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

### 2.10.2 INFRA-RED SPECTROSCOPY (IR)

A Shimadzu IR-470 spectrophotometer was used for recording infra-red spectrum. Major bands ($\nu_{max}$) were recorded in wave number ($cm^{-1}$) as KBr pellets.

### 2.10.3 NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPY

NMR spectra of pure sample were recorded by using 1H-NMR (400 MHz) and C-13 NMR spectrometer. The spectra were record *using* $CDCl_3$ *with tetramethyl silane* (TMS) as standard reference.

### 2.11 MELTING POINT APPARATUS

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

# 3. EXPERIMENTAL

# 3. EXPERIMENTAL

## 3.1 INVESTIGATION ON *PLEUROTUS OSTREATUS*

### 3.1.1 COLLECTION OF MUSHROOM

The mushroom "*Pleurotus Ostreatus*"have been collected from the National Mushroom Development and Extend Center, Savar of Dhaka district and these were identified from the Department of Botany, Dhaka University, The mushroom were dried under mild sunlight and then at$40^0$ C in an oven. Afterwards the mushroom were powdered in a grinding machine (~ 200 mesh). The powder was kept in airtight container and used throughout the investigation.

### 3.1.2 PHYTOCHEMICAL SCREENING OF THE MUSHROOM

Chemical tests were carried out on the aqueous extract and on the powdered specimens using standard procedures to identify the constituents[107-109]. Details of the experiment and results are furnished in the following sections.

### 3.1.3 QUALITATIVE DETERMINATION

**Test for flavonoids[110]:** Three methods were used to determine the presence of *flavonoids in the plant sample-*

**a)** Dilute ammonia solution (5 ml) was added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated $H_2SO_4$.

**b)** Few drops of aluminium ion ($Al^{3+}$) solution (1%) were added to a portion of each filtrate. A yellow coloration each observed indicating the presence of flavonoids.

**c)** A portion of the powdered plant sample each case was heated with ethyl acetate (10 ml) over a steam bath for 3 min. The mixture was filtered and the filtrate (4ml) was shaken with dilute ammonia solution (1 ml). A yellow colouration was not observed indicating a negative test for flavonoids.

**Test for steroids:** Acetic anhydride (2 ml) was added to ethanolic extract (0.5 g) of each sample with $H_2SO_4$ acid (2 ml). The colour changed from violet to blue or green in some samples indicating the presence of steroids.

**Test for terpenoids (Salkowski test):** *Each extract (5 ml) was mixed in chloroform* (2 ml), and concentrated $H_2SO_4$ acid (3 ml) was carefully added to form a layer. A reddish brown colouration at the interface was formed to show positive test for the presence of terpenoids.

**Test for cardiac glycosides (Keller-Killani test):** Each extracted (5 ml) was treated with glacial acetic acid (2 ml) containing ferric chloride solution (1 drop). This was underlayed with concentrated $H_2SO_4$ acid (1 ml). A brown ring at the interface indicates a deoxysugar characteristic of cardinolides. A violet ring may appear bellow the brown ring, while in the acetic acid layer, greenish ring may from just gradually throughout thin layer.

| | **Alkaloid** | **Flavonoid** | **Steroid** | **Terpenoid** | **Cardiac glycoside** |
|---|---|---|---|---|---|
| *Pleurotus Ostreatus* | + | - | + | + | + |

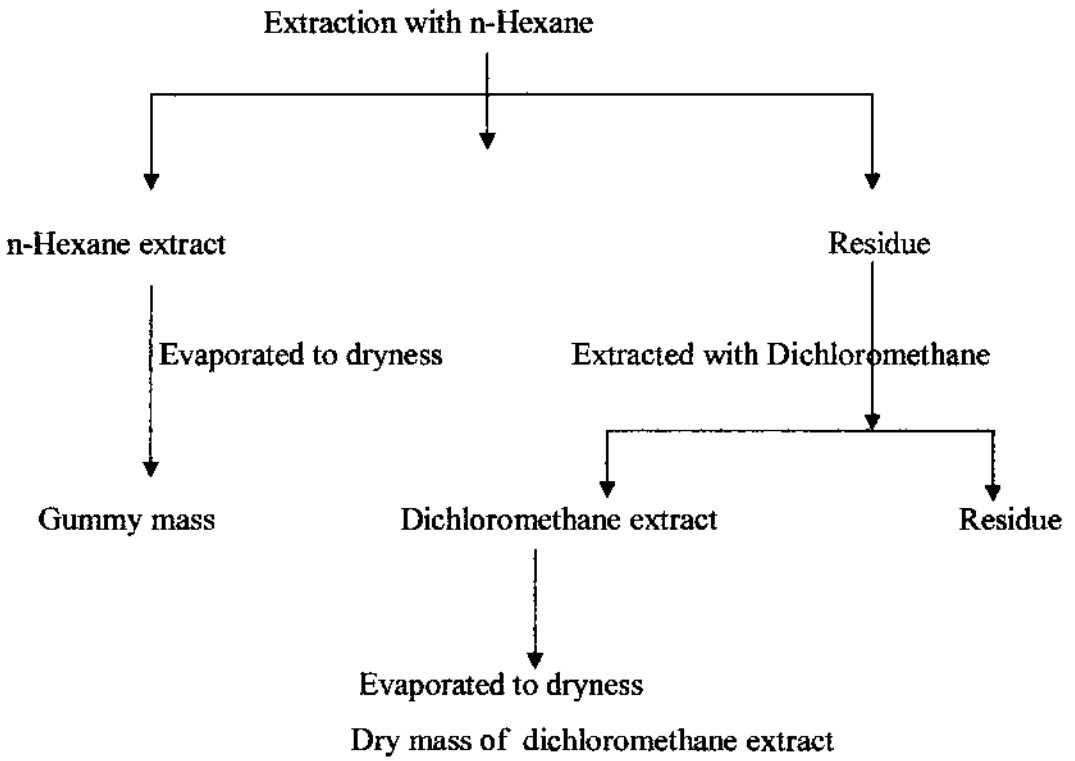
## 3.2. EXTRACTION AND ISOLATION OF COMPOUNDS FROM *PLEUROTUS OSTREATUS*

**Fig 3.1: Soxhlet apparatus**

Mushroom powder was taken in a few precleaned cloth thimbles. The thimbles containing the powder were placed in a Soxhlet apparatus.

The mushroom powder extracted separately and exhaustively in Soxhlet apparatus with n-hexane, dichloromethane and methanol. All the extracts were filtered individually; the filtrate was combined together and then concentrated in a "Buchi Rotavapor" under reduced pressure. This was shown in scheme-3.1

### 3.2.1 EXTRACTION SHEME OF *PLEUROTUS OSTREATUS*




Scheme 3.1: Extraction scheme of *Pleurotus Ostreatus*

**Table 3.1 Number of fraction collected in test-tubes from VLC of dichloromethane extract by using different solvent systems**

| Solvent systems | | | Amount of solvent (mL) | Number of test tubes |
|---|---|---|---|---|
| n-Hexane | Di-chloromethane | Methanol | | |
| 100% | 0 | 0 | 50 | 01-07 |
| 50% | 50% | 0 | 50 | 08-13 |
| 40% | 60% | 0 | 50 | 14-21 |
| 30% | 70% | 0 | 100 | 22-36 |
| 0 | 100% | 0 | 100 | 37-53 |
| 0 | 95% | 5% | 50 | 54-64 |
| 0 | 90% | 10% | 100 | 65-84 |
| 0 | 0 | 100% | 50 | 85-94 |

### 3.3.3 SCREENING OF THE FRACTIONS

TLC monitored each of the fractions and the fractions of similar behaviors were combined together and marked as F1, F2, F3, F4, F5, F6, F7, F8. This is given in Table- 3.2

**Table-3.2: Screening of the fractions by the similar TLC pattern**

| No of test tubes | Fractions | Solvent system for TLC | TLC pattern |
|---|---|---|---|
| 0-08 | F1 | 95:05 (DCM:MeOH) | Tailing |
| 09-10 | F2 | 95:05 (DCM:MeOH) | Two spots with tailing |
| 11-22 | F3 | 95:05 (DCM:MeOH) | Mixture |
| 23-33 | F4 | 100 (DCM) | Two spots |
| 34-55 | F5 | 95:05 (DCM:MeOH) | No spots |
| 56-58 | F6 | 90:10 (DCM:MeOH) | Mixture |
| 59-80 | F7 | 95:05 (DCM:MeOH) | Two spots with tailing |
| 81-94 | F8 | 90:10 (DCM:MeOH) | Mixture |

## 3.4 ATTEMPT OF PURIFICATION AND CHARACTERIZATION OF THE FRACTIONS

### 3.4.1 ANALYSIS OF FRACTION F1

The fraction F1 was light yellow in color. This was left undisturbed at room temperature for several days. It gave no crystals and gave an oily appearance. Then, the TLC study of the sample showed mixture on TLC plates on spraying with vanillin sulfuric acid followed by heating in an oven at $110^0$C for 10 minutes.

### 3.4.2 ANALYSIS OF FRACTION F2

The fraction F2 was concentrated to a small volume and was left undisturbed at room temperature. After several days a reddish semisolid substance was obtained at the bottom of the test tube. The TLC study of this showed two spots with tailing of pink colour on spraying with vanillin sulfuric acid followed by heating in oven at 110°C for 10 minutes. $R_f$ values of two sports are 0.67 and 0.56

### 3.4.3 ANALYSIS OF FRACTION F3

The fraction F3 left undisturbed at room temperature for several days and oily type substance was obtained. Its TLC study showed the presence of mixture with iodine and developing by vanillin sulfuric acid spray.

### 3.4.4 ANALYSIS OF FRACTION F4

Fraction F4 was left undisturbed at room temperature for several days. A white needle shaped crystal was obtained. The crystal were washed with solvents. After washing, the solid was dissolved in dichloromethane and the solution was kept in a vial for recrystallization at room temperature and crystals were obtained. Its TLC study also showed a single spot in solvent system , dichloromethane = 100. The spot was pink colour by spraying with vanillin sulfuric acid. The melting point of the compound is 122-124$^0$C and $R_f$ value is 0.76 .

Its TLC study also showed a single spot in different solvent systems. It was studied further.

### 3.4.5 ANALYSIS OF FRACTION F5

The fraction F5 left undisturbed at room temperature for several days. No crystal formed in this fraction and no distinct spot was observed in TLC study.

### 3.4.6 ANALYSIS OF FRACTION F6

The fraction F6 was concentrated to a small volume and was left undisturbed at room temperature. After several days an solid substance was obtained. The TLC study in solvent (DCM:MeOH=90:10) Showed the presence of two prominent bright spots. Solid was objected to column over column grade silica gel (kiesel gel 60, mesh 70-230). The column was first eluted with mixture of dicholoromethane and increasing amount of methanol. The eluents were collected in an amount of about 5mL in a series of test tubes. The solvent systems used for eluting the column were recorder below:

**Table 3.3: The solvent systems used successively for eluting the column (column with fraction F6)**

| Di-chloromethane | Methanol | Amount of solvent (mL) | Number of test tubes |
|---|---|---|---|
| 98% | 2% | 20 | 01-07 |
| 95% | 5% | 40 | 08-16 |
| 93% | 7% | 30 | 17-22 |
| 90% | 10% | 30 | 23-28 |
| 85% | 15% | 30 | 29-33 |
| 0 | 100% | 20 | 34-37 |

Each of the fractions was monitored by TLC (over silica gel PF254). Fractions of the similar TLC behavior were combined together and were designated as B-1(01-10); B-2(11-13); B-3(14-17); B-4(18-20); B-5(21-24); B-6(25-37).

**Table-3.4: Screening of the fractions by the similar TLC pattern**

| No. of test tubes | Fractions | Solvent system for TLC | TLC pattern |
|---|---|---|---|
| 01-10 | B1 | 95:05 (DCM:MeOH) | No spot |
| 11-13 | B2 | 95:05 (DCM:MeOH) | Two spots with tailing |
| 14-17 | B3 | 95:05 (DCM:MeOH) | One spot |
| 18-20 | B4 | 95:05 (DCM:MeOH) | No spot |
| 21-24 | B5 | 95:05 (DCM:MeOH) | Mixture |
| 25-37 | B6 | 95:05 (DCM:MeOH) | One spot |

### 3.4.7 ANALYSIS OF FRACTION B1

The fraction B1 left undisturbed at room temperature for several days. No crystal formed in this fraction and no distinct spot was observed in TLC study.

### 3.4.8 ANALYSIS OF FRACTION B2

The fraction B2 left undisturbed at room temperature for several days. After several days an reddish semisolid substance was obtained. The TLC study in solvent (DCM:MeOH=95:05) Showed the presence of two spots. Its paper chromatography studied.

### 3.4.9 ANALYSIS OF FRACTION B3

Fraction B3 was left undisturbed at room temperature for several days. A reddish crystal was obtained. The crystal were washed with solvents. The solid was dissolved in dichloromethane and the solution was kept in a vial for recrystallization at room temperature and crystals were obtained. Its TLC study also showed a single spot in solvent system , dichloromethane :methanol= 95:05. The spot was violet in colour by spraying with vanillin sulfuric acid and $R_f$ value is 0.66 .

Its TLC study showed a single spot in other solvents. It was studied further.

### 3.4.10 ANALYSIS OF FRACTION B4

The fraction B4 left undisturbed at room temperature for several days. No crystal formed in this fraction and no distinct spot was observed in TLC study.

### 3.4.11 ANALYSIS OF FRACTION B5

The fraction B5 left undisturbed at room temperature for several days and semisolid substance was obtained. Its TLC study showed mixture with iodine and developing by vanillin sulfuric acid spray.

### 3.4.12 ANALYSIS OF FRACTION B6

Fraction B6 was left undisturbed at room temperature for several days. A reddish crystal was obtained. The crystal were washed with solvents. The solid was dissolved in dichloromethane and the solution was kept in a vial for recrystallization at room temperature and crystals were obtained. Its TLC study also showed a single spot in solvent system , dichloromethane :methanol= 95:05. The spot was black in colour by spraying with vanillin sulfuric acid and $R_f$ value is 0.45.

Its TLC study also showed a single spot in different solvent systems. It was studied further.

### 3.4.13 ANALYSIS OF FRACTION F7

The fraction F7 left undisturbed at room temperature for several days and light yellow round type substance was obtained. Its TLC study showed two spots with iodine and vanillin sulfuric acid spray.

### 3.4.12 ANALYSIS OF FRACTION F8

The fraction F8 was reddish in colour. This was left undisturbed at room temperature for several days. It gave gummy type substance. Then, the TLC study of the samples showed mixture on TLC plates on spraying with vanillin sulfuric acid followed by heating in an oven for 10 minutes.

# 4. DETERMINATION OF ASH, MOISTURE, ACID VALUE, SAPONIFICATION VALUE, IODINE VALUD AND FATTY ACIDS

# 4. ANALYSIS OF ASH, MOISTURE, ACID VALUE, SAPONIFICATION VALUE, IODINE VALUE AND FATTY ACIDS

## 4.1 ANALYSIS OF ASH

### 4.1.1 INTRODUCTION

The "*ash content*[111]" is a measure of the total amount of minerals present within a food, whereas the "*mineral content*" is a measure of the amount of specific inorganic components present within a food, such as Ca, Na, K, Fe etc. Determination of the ash and mineral content of foods is important for a number of reasons.

*Nutritional labeling:* The concentration and type of minerals present must often be stipulated on the label of a food.

*Quality:* The quality of many foods depends on the concentration and type of minerals they contain, including their taste, appearance, texture and stability.

*Microbiological stability:* High mineral contents are sometimes used to retard the growth of certain microorganisms.

*Nutrition:* Some minerals are essential to a healthy diet (*e.g.*, calcium, phosphorous, potassium, sodium and iron) whereas others can be toxic (*e.g.*, lead, mercury, cadmium and aluminum).

### 4.1.2 DETERMINATION OF ASH CONTENT

Ash is the inorganic residue remaining after the water and organic matter have been removed by heating in the presence of oxidizing agents, which provides a measure of the total amount of minerals within a food. Analytical techniques for providing information about the total mineral content are based on the fact that the minerals (the "analyte") can be distinguished from all the other components (the "matrix") within a food in some measurable way. The most widely used methods are based on the fact that minerals are not destroyed by heating, and that they have a low volatility compared to other food components. The three main types of analytical procedure used to determine the ash content of foods are based on this principle: *dry* ashing, *wet* ashing and low temperature plasma dry ashing. The method chosen for a particular analysis depends on the reason for carrying out the analysis, the type of food analyzed and the equipment available. Ashing may also be used as the first step in preparing samples for analysis of specific minerals, by atomic spectroscopy or the various traditional methods described below. Ash contents of fresh foods rarely exceed 5%, although some processed foods can have ash contents as high as 12%, *e.g.,* dried beef.

### 4.1.3 DRY ASHING

Dry ashing procedures use a high temperature muffle furnace capable of maintaining temperatures of between 500 and 600°C. Water and other volatile materials are vaporized and organic substances are burned in the presence of the oxygen in air to $CO_2$, $H_2O$ and $N_2$. Most minerals are converted to oxides, sulfates, phosphates, chlorides or silicates. Although most minerals have fairly low volatility at these high temperatures, some are volatile and may be partially lost, *e.g., iron, lead and mercury. If an analysis is being* carried out to determine the concentration of one of these substances then it is advisable to use an alternative ashing method that uses lower temperatures.

The most widely used crucibles are made from porcelain because it is relatively inexpensive to purchase, can be used up to high temperatures (< 1200°C) and are easy to clean.

*Advantages:* Safe, few reagents are required, many samples can be analyzed simultaneously, not labor intensive, and ash can be analyzed for specific mineral content.

*Disadvantages:* Long time required (12-24 hours), muffle furnaces are quite costly to run due to electrical costs, loss of volatile minerals at high temperatures, *e.g.*, Cu, Fe, Pb, Hg, Ni, Zn.

Recently, analytical instruments have been developed to dry ash samples based on microwave heating. These devices can be programmed to initially remove most of the moisture (using a relatively low heat) and then convert the sample to ash (using a relatively high heat). Microwave instruments greatly reduce the time required to carry out an ash analysis, with the analysis time often being less than an hour. The major disadvantage is that it is not possible to simultaneously analyze as many samples as in a muffle furnace.

### 4.1.4 PROCEDURE

A weighed quantity of sample was taken in a crucible. The contents were heated carefully to the ignition point and allowed to burn spontaneously. When the burning was completed, the crucible heated at a dull-red heat in a muffle-furnace at 600°C for three hours. The crucible was then cooled and weighed with accuracy

### 4.1.5 CALCULATION

$$\text{Ash percentage} = \frac{100 \times W_2}{W_1}$$

Where, $W_1$ = Initial weight of the sample in g.

$W_2$ = Weight of the ash in g.

### 4.1.6 RESULT

The result of the determination of the amount of ash content of the plant powder of *pleurotus ostreatus* been calculated and found to be 14.89 and also presented in table-4.1

**TABLE-4.1: THE ASH CONTENT OF THE POWDER OF *PLEUROTUS OSTREATUS***

| Sample | Weight of crucible (g) | Weight of sample taken in the crucible $W_1$ (g) | Weight of crucible + ash (g) | Weight of ash obtained $W_2$ (g) | Percentage (%) |
|---|---|---|---|---|---|
| Powder of *Pleurotus ostreatus* | 28.4735 | 2.0018 | 28.4753 | 0.2981 | 14.89 |

### 4.1.7 DISCUSSION

The ash content of the sample was repeatedly checked the result was within the range of leafs, vegetables etc. The amount of ash contents of the mushroom that are locally used and conventional dietary intake, taken by the people of Bangladesh was investigated. Ash content measurement has been done to determine the important minerals in samples.

## 4.2 MOISTURE CONTENTS

### 4.2.1 INTRODUCTION

Sample can be preserved by drying. The longer the drying time, the less flavorful and the less tender the products are. The drying time can be hastened by drying small, uniformly cut pieces. Because they contain less acid than fruits, vegetables are dried until they are brittle. At this stage, only 10 percent moisture remains and no microorganisms can grow.

Controlled Moisture (CM) in vegetables combine superior performance and concentrated nutrients with vivid color and exceptional flavor.

CM Vegetables deliver better performance in frozen, fresh and refrigerated foods because they have less water content and less water loss than other frozen vegetable options.

With lower moisture and higher solid content, CM vegetables are nutritionally dense. In fact, it takes 33% less CM Vegetables in application to deliver one vegetable serving, compared to fresh and individually quick frozen (IQF) vegetables.

### 4.2.2 PREPARING SAMPLE

Only fresh sample in prime condition can produce a good-quality dried product. Wilted ones should not be used. One moldy bean may give a bad flavor to an entire lot. The sample are gathered early in the morning, and started the drying process as soon as possible. Carefully sorted, discarding any bruised or undesirable product. Trim, peel, cut, slice or shred sample. Keep pieces uniform in size so they will dry at the same rate. Holding sample, even in the refrigerator and preparation for drying will result in loss of quality and nutrients.

### 4.2.3 PROCEDURE

The mushroom powder was taken in a weighing bottle and placed in a oven at100 °C for several days .The weighing bottle containing sample was weighed after cooling at room temperature in dessicator. Again it was placed in oven and weighed after cooling in several times. When a constant weight was found, it was recorded.

### 4.2.4 RESULT

The result of the determination of moisture content of the mushroom powder of *pleurotus ostreatus* is given in table.

**TABLE-4.2: THE MOISTURE CONTENT OF THE MUSHROOM POWDER OF *PLEUROTUS OSTREATUS***

| Sample | Weight of weighing bottle (g) | Weight of sample taken in the weighing bottle, $W_1$ (g) | Weight of weighing bottle after loosing moisture (g) | Weight of moisture obtained $W_2$ (g) | Percentage (%) |
|---|---|---|---|---|---|
| Mushroom powder of *pleurotus ostreatus* | 17.1131 | (22.1942-17.1131) = 5.0811 | 21.7521 | 4.639 | 8.70 |

### 4.2.5 DISCUSSION

The air dried mushroom contained 8.7% moisture content which is little lower than other type of plant material usually moisture content is such plant and dried vegetable material is 10-12% w/w. The moisture contents in the samples are also important. The relative recommended moisture level in sample helps to digest food properly. Sample contain considerable percentage of water. This water part has several minerals and nutrients that help to develop the resistant power of diseases inside our body

## 4.3 DETERMINATION OF ACID VALUE

Acid value indicates the amount of free fatty acid in the oil or fat and may be defined as the number of milligrams of caustic potash required to neutralize the trace acid in one gm of the sample. The normal acid value for most samples lies within 0.5. If any titrable acid other than a fatty acid is present in the sample. It will be an error.

### 4.3.1 PROCEDURE

Weight 1-2 g of n-hexane extract into a 250ml. conical flask and 50 ml.of 95% alcohol. Heat the contents to boiling and shake the flask thoroughly, in order to dissolve the free acids as completely as possible. The solution is cooled and then titrated against 0.01 M alcoholic caustic potash solution with constant shaking ( using phenolphthalein indicator ) until the prink colour persists after vigorous shaking. For good result, always keep the solution hot, even if no precipitation occurs (oils containing much fatty acids when titrated with alkali give soaps that precipitate on cooling and occlude some of the solution ). Heating makes the soap soluble so that titration may not be complicated. End point is supposed to be reached when the pink colour persists for 30 seconds in the hot solution after vigorous shaking. If the sample in largely fatty acid, satisfactory results are obtained with 1-2 g of the sample. A blank experiment is conducted following the identical procedure with the omission of the extract. The difference between the readings provides the amount of KOH consumed.

### 4.3.2 RESULT

Amount of n-Hexane extract taken = 0.2730g

**Table4.3**: Titration of free fatty acid content in n-Hexane extract with standard 0.01M KOH solution using pheolophthalein indicator

| Observation no. | Burette reading (mL) | | Volume of KOH (mL) |
|---|---|---|---|
| | Initial | Final | |
| 1 | 0.0 | 19.1 | 19.1 |
| BLANK | 0.0 | 41.5 | 41.5 |

The amount of KOH used = (41.5-19.1) mL =22.4 mL

Moles of KOH = $22.4 \times 10^{-3} \times 0.01 \times 56$

= 12.544 mg

For nutraligation,

0.2730 g n-Hexane extract requires 12.544mg KOH

$$1 \text{ g} \quad \text{„} \quad \text{„} \quad \frac{12.544 \times 1.0}{0.2730}$$

= 45.94 mg KOH

**Result**: Acid value of n-Hexane extract is 45.94

## 4.4 DETERMINATION OF SAPONIFICATION VALUE

Saponification value indicates the average molecular weight of a fat or oil. The saponification value may be defined as the number of milligrams of caustic potash required to neutralize the fatty acids obtained by complete hydrolysis of one gm of oil or fat. Thus a saponification value gives us information, whether :

a)An oil or fat contains lower and higher proportion of some acids.

b)An oil or fat contains high proportion of lower or higher fatty acids.

Since butter has a large proportion of lower fatty acids than lard and tallow, the former has high saponification value .

Coconut oil has a comparatively higher saporification value (250) than olive oil (200) and castor oil (180). Since these contains glycosides of higher proportion of relatively high molecular weight fatty acids.

### 4.4.1 PROCEDURE

2.0 gms of n-hexane extract was weighted into a flask and 25ml of alcoholic solution KOH (30 mgs KOH in a minimum volume of water and diluted to 1.0 liter with 96% alcohol) was added by pipette. The flask was connected to reflux condensed and the mixture was boiled for an at least half an hour over a water bath with occasional shaking (when saponification was complete the mixture became homogeneous and no drops of fat remained unreacted).The contents of the flasks were cooled and unreacted alkali was titrated with standard HCL using phenolphthalein indicator. A blank experiment under identical conditions was performed with the omission of oil.

### 4.4.2 RESULT

**Experimental data for determination of saponification value**

Amount of n-Hexane extract taken =0.1722g

**Table-4.4:** n-Hexane extract and blank titration of iodine with standard 0.5M HCl.

| Observation No. | Sample | Initial burette reading (ml) | Final burette reading (ml) | Volume of HCl added (ml). |
|---|---|---|---|---|
| 1 | n-Hexane extract | 1.0 | 18.5 | 17.5 |
| 2 | Blank | 0.0 | 19.1 | 19.1 |

The amount of alkali used in saponification = (19.1-17.5) mL

=1.6 mL

Moles of HCl = 0.5M ×1.6 ×$10^{-3}$ L

= 0.8 ×$10^{-3}$ moles

Moles of KOH = 0.8 ×$10^{-3}$ moles

Amount of KOH = 0.8 ×$10^{-3}$ × 56 g

= 44.8 × $10^{-3}$ g

0.1722g n-Hexanereact with 44.8 × $10^{-3}$ g KOH

$$1 \text{ g } \text{„} \quad \text{„} \quad \frac{44.8 \times 10^{-3}}{0.1722} \text{ g KOH}$$

= 260.16 mg

**Result**: Saponification value of the n-Hexane extract is 260.16

## 4.5 DETERMINATION OF IODINE VALUE

Iodine value shows the degree of unsaturation of the constituent fatty acids in an oil or fat and in thus a relative measure of the unsaturated bonds present in the oil or fat. Iodine value in expressed in grams of iodine absorbed by 100 gms of oil or fat. Unsaturated compounds absorb iodine (in suitable form) and form saturated compounds. The amount of iodine absorbed in percentage is the measure of unsatusation in the oil. No oil has zero iodine value and oils are classified as drying, semidrying and non-drying on the basis of iodine value . Non-drying oils have one double bond have iodine value approximately 90. Semidrying oils contain some proportion of double bonds and have iodine value below 140. Oils containing some proportion of triple bond and having iodine value above 140, are called drying oils. The iodine value for coconut oil 8, for olive oil 88, for human fat is105 and for linseed oil about 200.

### 4.5.1 PROCEDURE

10 drops of n-hexane extract was weighted in a well stoppered bottle. The oil was dissolved in 10 ml of chloroform or carbon tetrachloride. 25 ml of Henus solution was added from a burette and allowed to stand for exactly half an hour with occasional shaking. 100 ml of water was added at the end of this period followed by 10 ml of 12% KI solution and the iodine left unreacted was titrated with standard thiosulfate using starch indicator. A blank experiment was performed with the omission of oil. The difference between the volumes of thiosulfate required corresponds to the amount of iodine absorbed by the oil.

### 4.5.2 RESULT

Experimental data for determination of iodine value

Amount of n-Hexane extract taken =0.2507g

**Table-4.5: n-Hexane extract and blank titration of iodine with standard 0.1M $Na_2S_2O_3$.**

| Observation no. | Sample | Initial burette reading (ml) | Final burette reading (ml). | Volume of thiosulfate added (ml). |
|---|---|---|---|---|
| 1 | n-Hexane extract | 0.0 | 45.0 | 45.0 |
| 2 | Blank | 0.0 | 76.2 | 76.2 |

Volume of required to titrate consumed iodine by the n-Hexane extract = (76.2-45.0) = 31.2 mL

Moles of $Na_2S_2O_3$ =0.1M × 31.2 × $10^{-3}$L

=3.12 × $10^{-3}$ moles

1 moles = mole $Na_2S_2O_3$

Amount of $I_2$ = × 3.12 × $10^{-3}$ ×127 × 2

=396.24 × $10^{-3}$ g

0.2507 g n-Hexane liberated 396.24 × $10^{-3}$ g $I_2$

$$100 \text{ g} \quad \text{„} \quad \frac{396.24 \times 10^{-3} \times 100}{0.2507}$$

$$= 158.05 \text{ g}$$

**Result**: The iodine value of the n-Hexane extract is 158.05

## 4.6 PROCEDURE OF FATTY ACID ANALYSIS

### 4.6.1 ANALYSIS OF FATTY ACIDS[112]

n-hexane extract (2.25g) of *Pleurotus Ostreatus* was dissolved in n-hexane (50 ml) and extracted with 5% sodium bicarbonate solution (25 ml x 2).The mixture was taken in a seperatory funnel and shaken vigorously and allowed to stand for overnight. Two layers were obtained. The lower layer (aqueous) was separated and taken for the analysis of free fatty acid (FFA). The upper layer was separated and taken for the analysis of bound fatty acid (BFA).

### 4.6.2 ISOLATION OF FFA:

2.25 g dry n-Hexane extract was saponified and the saponification material was esterified with borontrifluoride-methanol complex.

### 4.6.3 SAPONIFICATION

500 mg dry sample n-hexane extract was taken in a pear-shaped flask and 2.0 mL 0.5M methanolic NaOH was added to it. It was shaked well and refluxed for 30 minutes in a boiling water bath at $100^0$C. The mixture was evaporated and 5.0 mL water was added to it. pH of the solution was kept at 2.5 and extracted with hexane. The hexane part was taken in a conical flask and anhydrous $Na_2SO_4$ was added to it to make it completely free from water. The solution was then filtered and the hexane part was dried. This sample contains the constituent fatty acids and was used for esterification.

### 4.6.4 ESTERIFICATION

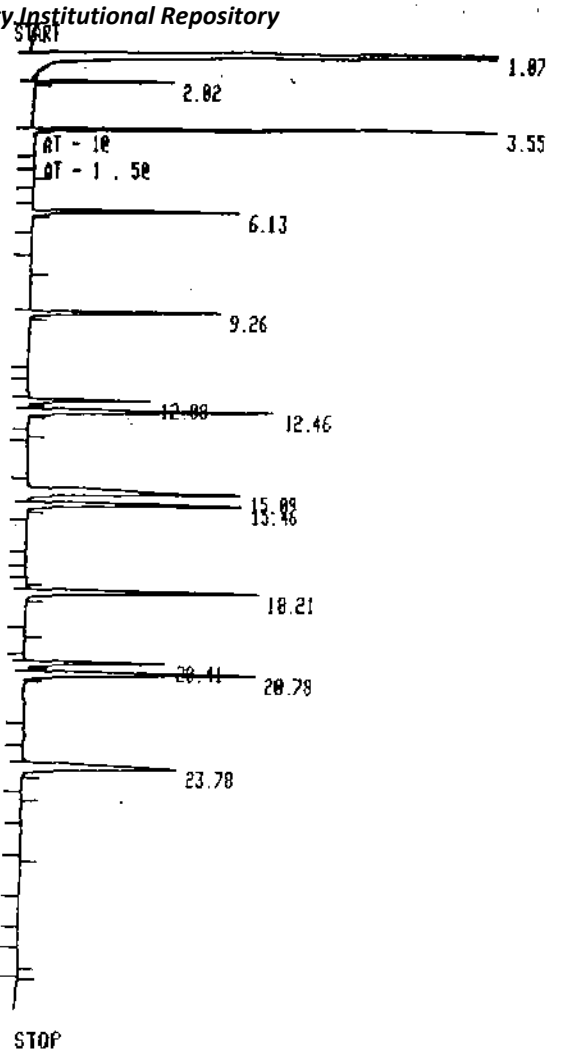
2.0 mL $BF_3$-MeOH complex was added to the saponified material as obtained in the previous experiment in a pear-shaped flask. It was refluxed for 10 minutes in a boiling water bath. The mixture was evaporated to dryness in rotary evaporator and transferred in a separototy funnel containing a little water. The content was shaken vigorously and then hexane was added to it and hexane part was taken in a small vial for analyzing in GC. (Shimadzu 9A, Column-BP-50, Detector-FID, 170°C-1 min/4°C-270°C-30 min).

### 4.6.5 IDENTIFICATION OF FATTY ACIDS

By measuring the retention times of the fatty acids in the experimental sample and comparing them with those of the standard reference methyl esters the following fatty acids were identified.

**Table-4.6: Study of different fatty acids in n-hexane extract from *pleurotus ostreatus***

| Name of the fatty acids | Retention time of standard acids (Min) | Retention time of experimental acids (Min) | Relative percentage of fatty acids |
|---|---|---|---|
| Capric acid | 2.05 | 2.05 | 14.36 |
| Lauric acid | 9.27 | 9.27 | Trace |
| Palmitic acid | 12.70 | 12.75 | 38.73 |
| Oleic acid | 15.36 | 15.36 | 37.40 |
| Stearic acid | 15.61 | 15.61 | 7.40 |



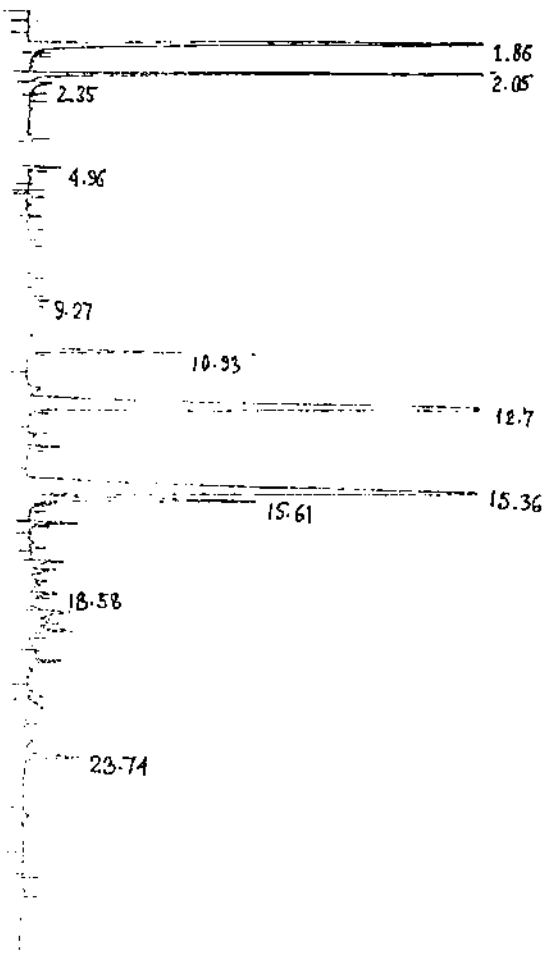

RUN # 5

AREA%

| RT | AREA | TYPE | AR/HT | AREA% |
|---|---|---|---|---|
| 1.07 | 1715380 | PB | 0.047 | 93.739 |
| 2.82 | 1193 | PB | 0.033 | 0.0[illegible] |
| 3.55 | 5903 | PB | 0.047 | 0.323 |
| 6.13 | 7428 | PB | 0.058 | 0.4[illegible] |
| 9.26 | 8085 | PB | 0.081 | 0.442 |
| 12.08 | 5156 | PB | 0.079 | 0.2[illegible] |
| 12.46 | 12406 | BB | 0.095 | 0.67[illegible] |
| 15.09 | 16718 | PB | 0.149 | 0.9[illegible] |
| 15.46 | 11118 | BB | 0.098 | 0.607 |
| 18.21 | 12982 | BB | 0.106 | 0.710 |
| 20.41 | 6208 | BB | 0.085 | 0.339 |
| 20.78 | 13471 | BB | 0.111 | 0.736 |
| 23.78 | 13919 | BB | 0.173 | 0.761 |

TOTAL AREA= 1829800
MUL FACTOR= 1.0000E+00

FIG 4 I SEPARATION OF FATTY ACID BY GAS LIQUID CHROMATOGRAPHY (STANDARD)




| | | | | |
|---|---|---|---|---|
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| 19.18 | 2442 | BV | 0.121 | 9.34 |
| 19.78 | 1153 | BB | 0.066 | 0.17 |
| 23.74 | 3146 | BB | 0.198 | 0.44 |

TOTAL AREA= 708820
MUL FACTOR= 1.0000E+00

FIG 4.2 SEPARATION OF FATTY ACID BY GAS LIQUID CHROMATOGRAPHY (SAMPLE)

### 4.6.6 DISCUSSION

n-Hexane extract of mushroom which usually contains non polar substances like lipids, sterols, waxes, plant hydrocarbons etc. The free fatty acid content in this extract was determined. By determining the acid value, which reflects the presence of free fatty acids in the extract. The acid value was found to be 45 determined as the number of milligrams of KOH consume by each gram of the extract sample.

Saponification value and iodine value of the n-hexane extract was determined and results were found to be 260 and 158 respectively.

The Saponification value reflect the distribution of long chain carboxylic acids specially their molecular weight while the iodine value measured the unsaturation of the extract. The iodine value of the extract falls within the range of polyunsaturated oils which is highly recommended for edible use. The presence of smaller fatty acids is reflected by its saponification value which is comparable to that of coconut oil and is good stuff for making special type of soap.

The constituent fatty acids the fat present in the n-hexane extract was determined by GLC as the methyl ester of the constituent fatty acids.Fat was saponified and the constituent fatty acids were recorded by extracting with n-hexane and was subjected to esterification with boron trifluoride methanol complex, a good method for converting the acids into their volatile methyl ester derivatives.

Fat is an important component of our diet and supports the body processes. Dietary fats are classified by their structure. Different types of fats react differently inside the body. Saturated fats (found mostly in animal products) increase blood cholesterol, which is a risk factor in coronary heart disease. Unsaturated fats (found mostly in plant and marine foods) tend to lower blood cholesterol. The compositional analysis of petroleum ether extract of *Pleurotus Ostreatus* by GC revealed that the bound fatty acid portion of both the n-hexane extract of *Pleurotus Ostreatus* were rich in unsaturated fatty acids (such as Oleic acid). Thus it can be concluded that mushroom consists of fatty matter which are rich in unsaturated constituents and are within recommended dietary fatty acids.

# 5. RESULTS AND DISCUSSION

# 5. RESULTS AND DISCUSION

The spcies *Pleurotus ostreatus* (oyster mushroom) of the family Pleurotaceae has been collected from the National Mushroom Development and Extend Center, Savar of Dhaka district and these were identified from the department of Botany, Dhaka University, The mushroom were dried under mild sunlight and then at $40^0$ C in an oven. Afterwards the mushrooms were powdered in a grinding machine (~ 200 mesh) and extracted with n-hexane followed by dichloromethane. Dichloromethane extract of this species was fractionated by VLC (vacuum liquid chromatography) & different fractions obtained were separated & purified using different chromatographic techniques. The compounds isolated from this extract were characterized by different physical and spectroscopic studies.

## 5.1 CHARACTERISATION OF COMPOUNDS ISOLATED FROM DICHLOROMETHANE EXTRACT OF *PLEUROTUS OSTREATUS*

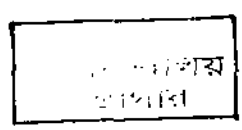
### 5.1.1 CHARACTERISATION OF COMPOUND F4

The compounds F4 (78.9 mg) was isolated as white crystalline powder (melting point 122-124$^0$C) from the dichloromethane extract of the oyster mushroom *pleurotus ostreatus*. It was soluble in dichloromethane. TLC examination of F4 indicated that it was a single compound $R_f$ value = 0.76 (over silica gel, $GF_{254}$, petroleum ether: dichloromethane = 90:10 as the mobile phase). It was visualized as a brownish coloured spot upon its exposure to iodine vapour and as a violet coloured spot on sparaying with vanillin-sulfuric acid followed by heating in an oven at 110°C.

## 5.1.2 IR SPECTROSCOPIC ANALYSIS OF COMPOUND F4

The IR[113] spectrum(Fig 5.1.1) of the compound F4 showed an absorption band at (3500-3400) $cm^{-1}$ indicating the presence of –OH group. The band at 2950.9, 1458.3, 1371.3 $cm^{-1}$ was due to the presence of aliphatic C-H stretching of either both $–CH_3$, $-CH_2-$ or >CH- group. The peak at 1650 $cm^{-1}$ was suggestive the presence of >C=C< group. The peak at 1458.1 $cm^{-1}$ was due to the presence of $–CH_3$ group. The other peak at 970.1 $cm^{-1}$ for alkene (out of plane bend) and 1028, 1055 $cm^{-1}$ were due to C-O stretching vibration.





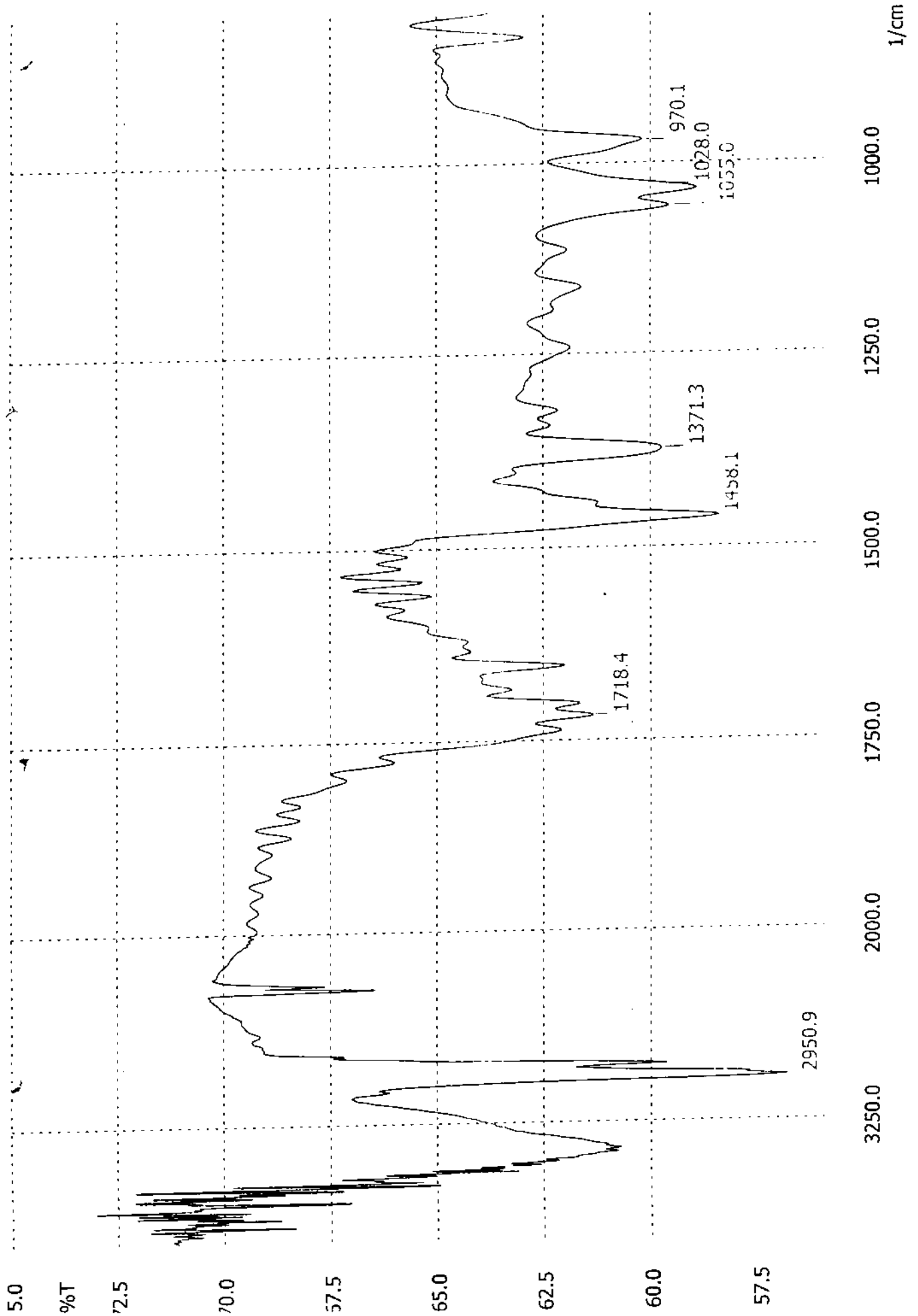



FIG 5.1.1 IR SPECTRUM OF COMPOUND F4

### 5.1.3 $^1$H-NMR SPECTROSCOPIC ANALYSIS OF COMPOUND F4

The $^1$H-NMR[114] (400MHz in $CDCl_3$) spectrum (Fig-5.1.2-5.1.6) of the isolated compound F4 showed a downfield broadband multiplet at δ 5.13-5.55 ppm suggestive of the presence of olefinic proton (6-H and 7-H).

The olefin protons H-22 and H-23 appeared as characteristics downfield signal at δ 5.17 and δ 5.21 ppm respectively in the $^1$H NMR spectrum. These two signals were observed as multiplet which indicated the presence of characteristics olefinic protons.

The $^1$H NMR spectrum displayed two three-proton singlets at δ 0.937 and δ 0.622 ppm assignable to two angular methyl groups at C-19 and C-18 respectively.

The spectrum showed a broadened multiplet at δ 3.59-3.62 ppm suggestive of an oxymethine proton 3-H.

In addition, three doublets at δ 0.823 (J = 6.9 Hz), 0.839 (J = 7.0 Hz) and 0.90 (J=6.8 Hz) integrating for three protons to the three methyl group at C-26, C-27 and C-28. Another three-proton doublet at δ 0.91 (J = 6.4 Hz) could be attributed to the methyl group at C-21. These spectral features are characteristics of the following steroidal carbon skeleton of F4.

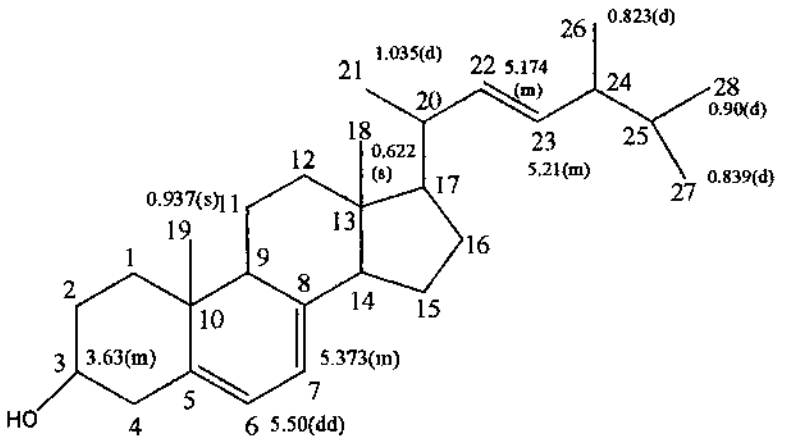


**Table-5.1: $^1$H-NMR spectral data of compound F4**

| No.of protons | Chemical shift value(δ) | |
|---|---|---|
| | Experiment value(δ) | Reported value[115] (δ) |
| H-6 | 5.50(dd) | 5.56(dd, J=5.8 Hz) |
| H-7 | 5.373(m) | 5.38( m) |
| H-23 | 5.21(m) | 5.21(m) |
| H-22 | 5.174(m) | 5.17( m) |
| 1H-3a | 3.622(bm) | 3.63(bm) |
| 21-$H_3$ | 1.035(d) | 1.04(d, J= 6.6 Hz) |
| 19-$H_3$ | 0.937(s) | 0.94(s) |
| 28-$H_3$ | 0.90(d) | 0.90(d, J= 6.8 Hz) |
| 27-$H_3$ | 0.839(d) | 0.84(d, J= 7.0 Hz) |
| 26-$H_3$ | 0.823 (d) | 0.82 (d, J=6.9 Hz) |
| 18- $H_3$ | 0.622 (s) | 0.62 (s) |

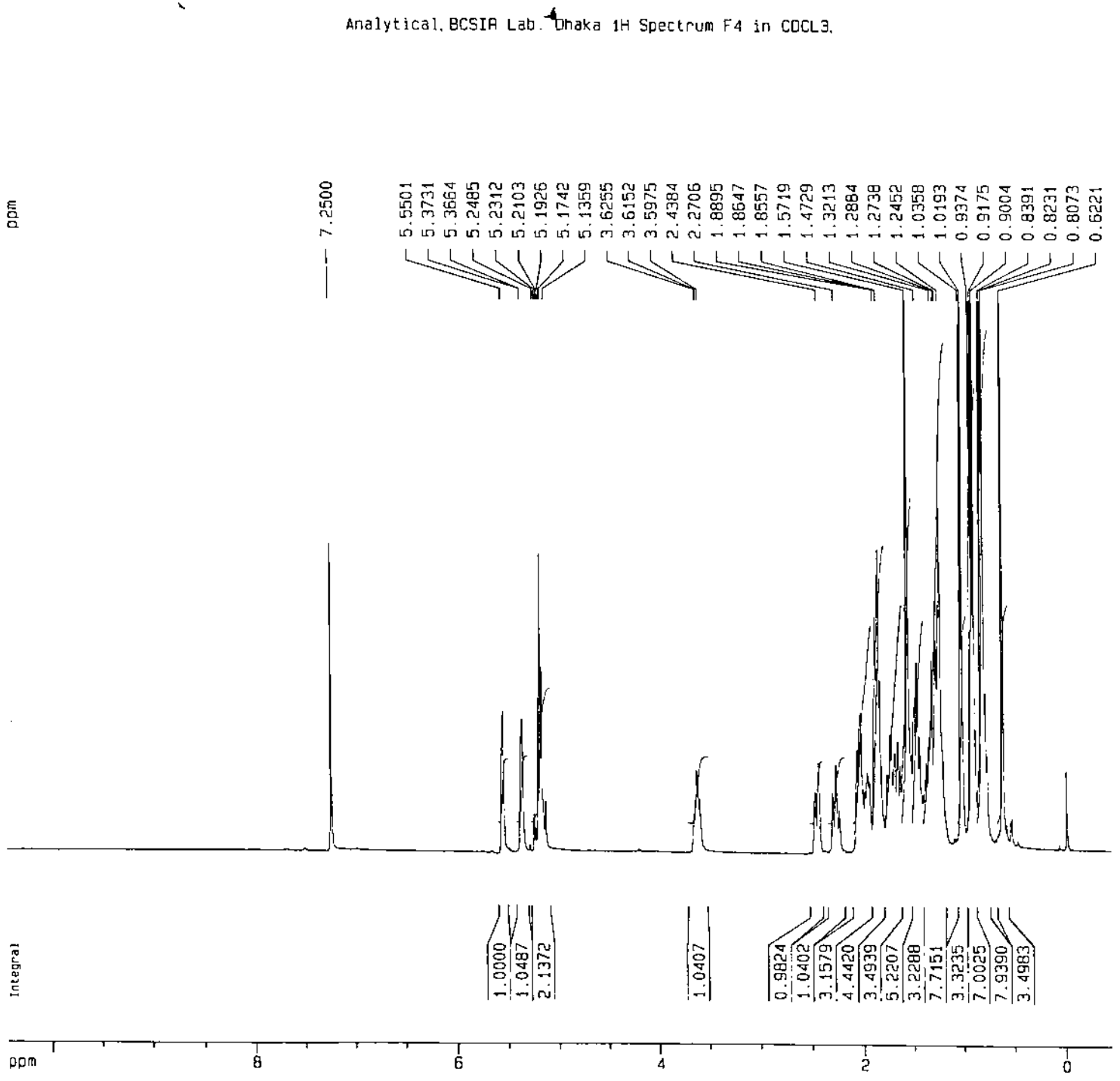



FIG 5.1.2 $^{1}$H-NMR SPECTRUM OF COMPOUND F4

Current Data Parameters

| | |
|---|---|
| NAME | A4599 |
| EXPNO | 1 |
| PROCNO | 1 |

F2 - Acquisition Parameters

| | |
|---|---|
| Date_ | 20080903 |
| Time | 12.09 |
| INSTRUM | dpx400 |
| PROBHD | 5 mm Multinuc |
| PULPROG | zg30 |
| TD | 32768 |
| SOLVENT | CDCl3 |
| NS | 128 |
| DS | 2 |
| SWH | 6410.256 Hz |
| FIDRES | 0.195625 Hz |
| AQ | 2.5559540 sec |
| RG | 181 |
| DW | 78.000 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 | 400.1428010 MHz |

F2 - Processing parameters

| | |
|---|---|
| SI | 32768 |
| SF | 400.1400126 MHz |
| WDW | EM |
| SSB | 0 |
| LB | 0.30 Hz |
| GB | 0 |
| PC | 1.40 |

1D NMR plot parameters

| | |
|---|---|
| CX | 20.00 cm |
| F1P | 10.450 ppm |
| F1 | 4181.42 Hz |
| F2P | -0.446 ppm |
| F2 | -178.37 Hz |
| PPMCM | 0.54478 ppm/cm |
| HZCM | 217.98959 Hz/cm |

Analytical, BCSIR Lab. Dhaka 1H Spectrum F4 in CDCL3.

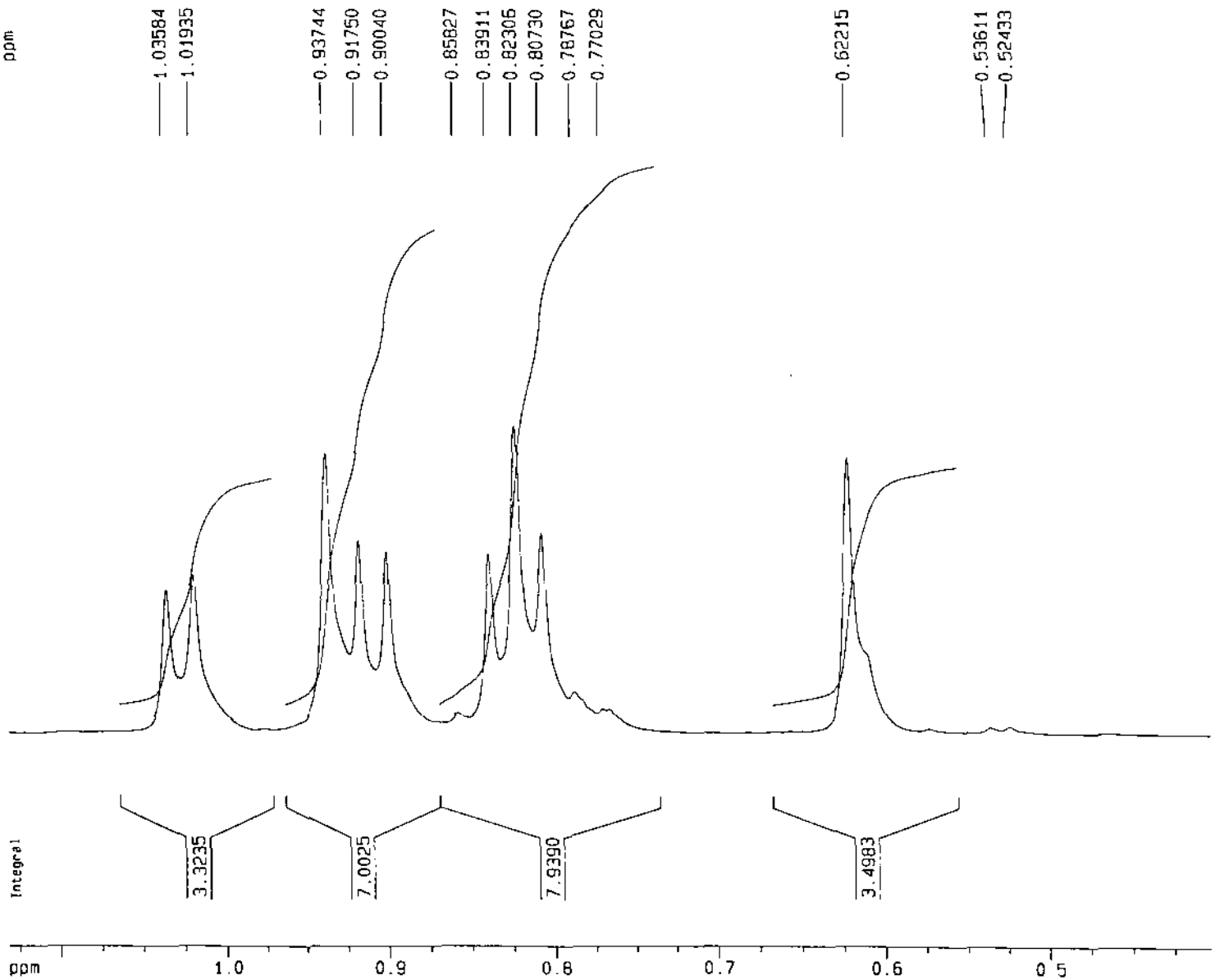



FIG 5.1.3 EXPANDED $^{1}$H-NMR SPECTRUM OF COMPOUND F4

Current Data Parameters

| | |
|---|---|
| NAME | A4599 |
| EXPNO | 1 |
| PROCNO | 1 |

F2 - Acquisition Parameters

| | |
|---|---|
| Date_ | 20080903 |
| Time | 12.09 |
| INSTRUM | dpx400 |
| PROBHD | 5 mm Multinuc |
| PULPROG | zg30 |
| TD | 32768 |
| SOLVENT | CDCl3 |
| NS | 128 |
| DS | 2 |
| SWH | 6410.256 Hz |
| FIDRES | 0.195625 Hz |
| AQ | 2.5559540 sec |
| RG | 181 |
| DW | 78.000 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 | 400.1428010 MHz |

F2 - Processing parameters

| | |
|---|---|
| SI | 32768 |
| SF | 400.1400126 MHz |
| WDW | EM |
| SSB | 0 |
| LB | 0.30 Hz |
| GB | 0 |
| PC | 1.40 |

1D NMR plot parameters

| | |
|---|---|
| CX | 20.00 cm |
| F1P | 1.132 ppm |
| F1 | 453.11 Hz |
| F2P | 0.403 ppm |
| F2 | 161.15 Hz |
| PPMCM | 0.03648 ppm/cm |
| HZCM | 14.59804 Hz/cm |

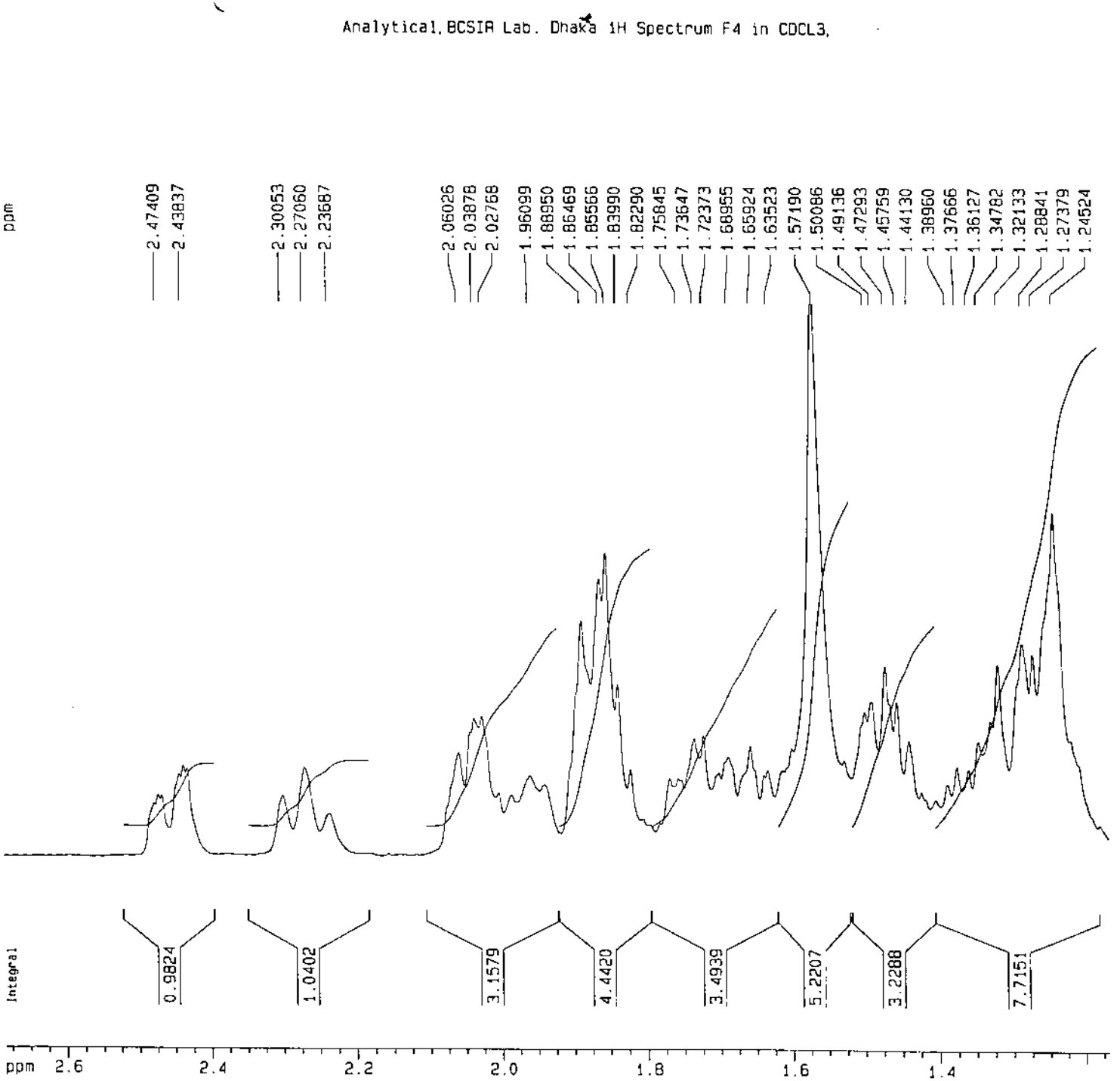



FIG 5.1.4 EXPANDED $^1$H-NMR SPECTRUM OF COMPOUND F4

Current Data Parameters

| | |
|---|---|
| NAME | A4599 |
| EXPNO | 1 |
| PROCNO | 1 |

F2 - Acquisition Parameters

| | |
|---|---|
| Date_ | 20080903 |
| Time | 12.09 |
| INSTRUM | dpx400 |
| PROBHD | 5 mm Multinuc |
| PULPROG | zg30 |
| TD | 32768 |
| SOLVENT | CDCl3 |
| NS | 128 |
| DS | 2 |
| SWH | 6410.256 Hz |
| FIDRES | 0.195625 Hz |
| AQ | 2.5559540 sec |
| RG | 181 |
| DW | 78.000 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 | 400.1428010 MHz |

F2 - Processing parameters

| | |
|---|---|
| SI | 32768 |
| SF | 400.1400126 MHz |
| WDW | EM |
| SSB | 0 |
| LB | 0.30 Hz |
| GB | 0 |
| PC | 1.40 |

1D NMR plot parameters

| | |
|---|---|
| CX | 20.00 cm |
| F1P | 2.686 ppm |
| F1 | 1074.69 Hz |
| F2P | 1.168 ppm |
| F2 | 467.35 Hz |
| PPMCM | 0.07589 ppm/cm |
| HZCM | 30.36703 Hz/cm |

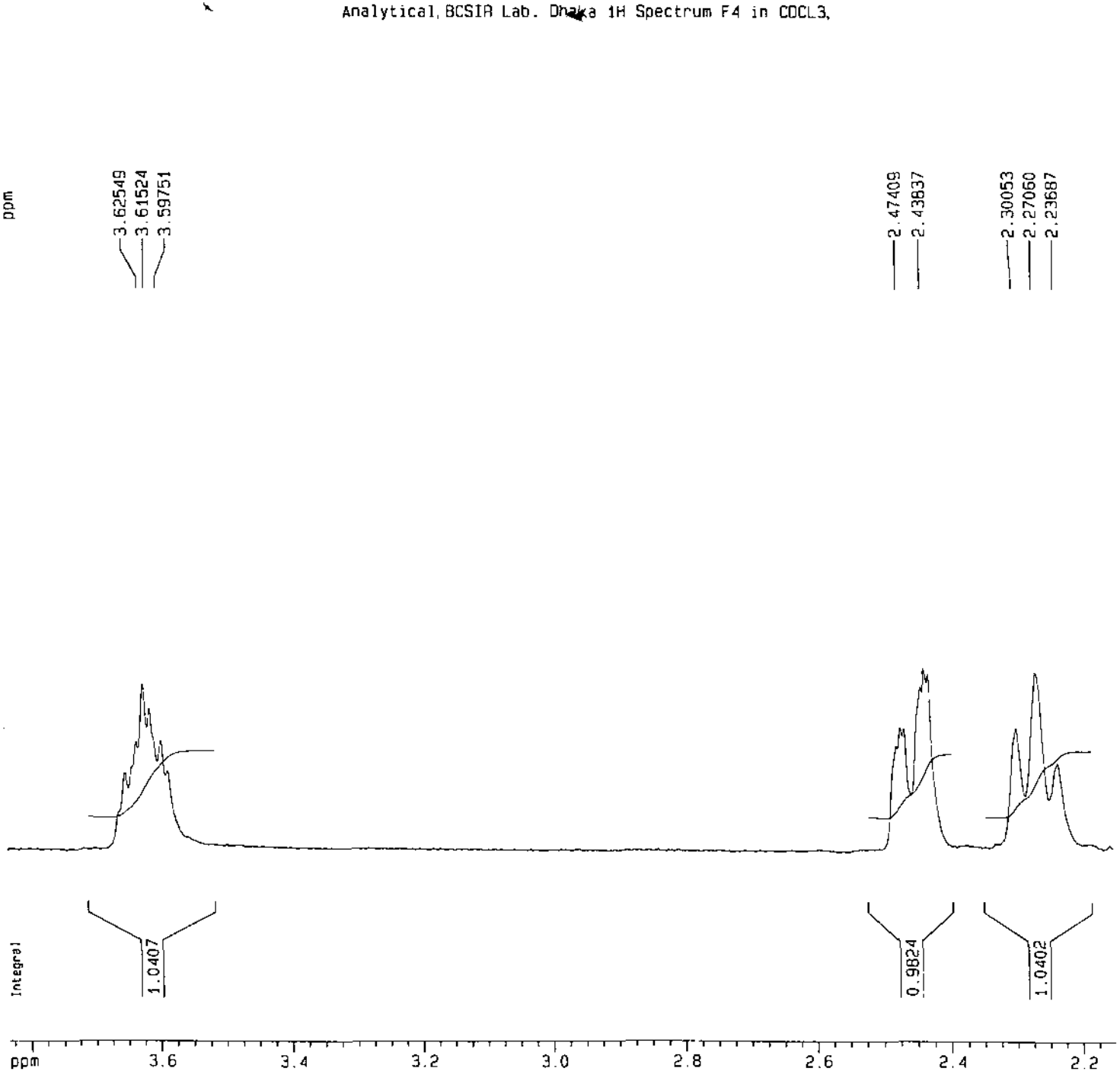



FIG 5.1.5 EXPANDED $^{1}$H-NMR SPECTRUM OF COMPOUND F4

Current Data Parameters

| | |
|---|---|
| NAME | A4599 |
| EXPNO | 1 |
| PROCNO | 1 |

F2 - Acquisition Parameters

| | |
|---|---|
| Date_ | 20080903 |
| Time | 12.09 |
| INSTRUM | dpx400 |
| PROBHD | 5 mm Multinuc |
| PULPROG | zg30 |
| TD | 32768 |
| SOLVENT | CDCl3 |
| NS | 128 |
| DS | 2 |
| SWH | 6410.256 Hz |
| FIDRES | 0.195625 Hz |
| AQ | 2.5559540 sec |
| RG | 181 |
| DW | 78.000 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 | 400.1428010 MHz |

F2 - Processing parameters

| | |
|---|---|
| SI | 32768 |
| SF | 400.1400126 MHz |
| WDW | EM |
| SSB | 0 |
| LB | 0.30 Hz |
| GB | 0 |
| PC | 1.40 |

1D NMR plot parameters

| | |
|---|---|
| CX | 20.00 cm |
| F1P | 3.834 ppm |
| F1 | 1533.95 Hz |
| F2P | 2.151 ppm |
| F2 | 860.79 Hz |
| PPMCM | 0.08412 ppm/cm |
| HZCM | 33.65837 Hz/cm |

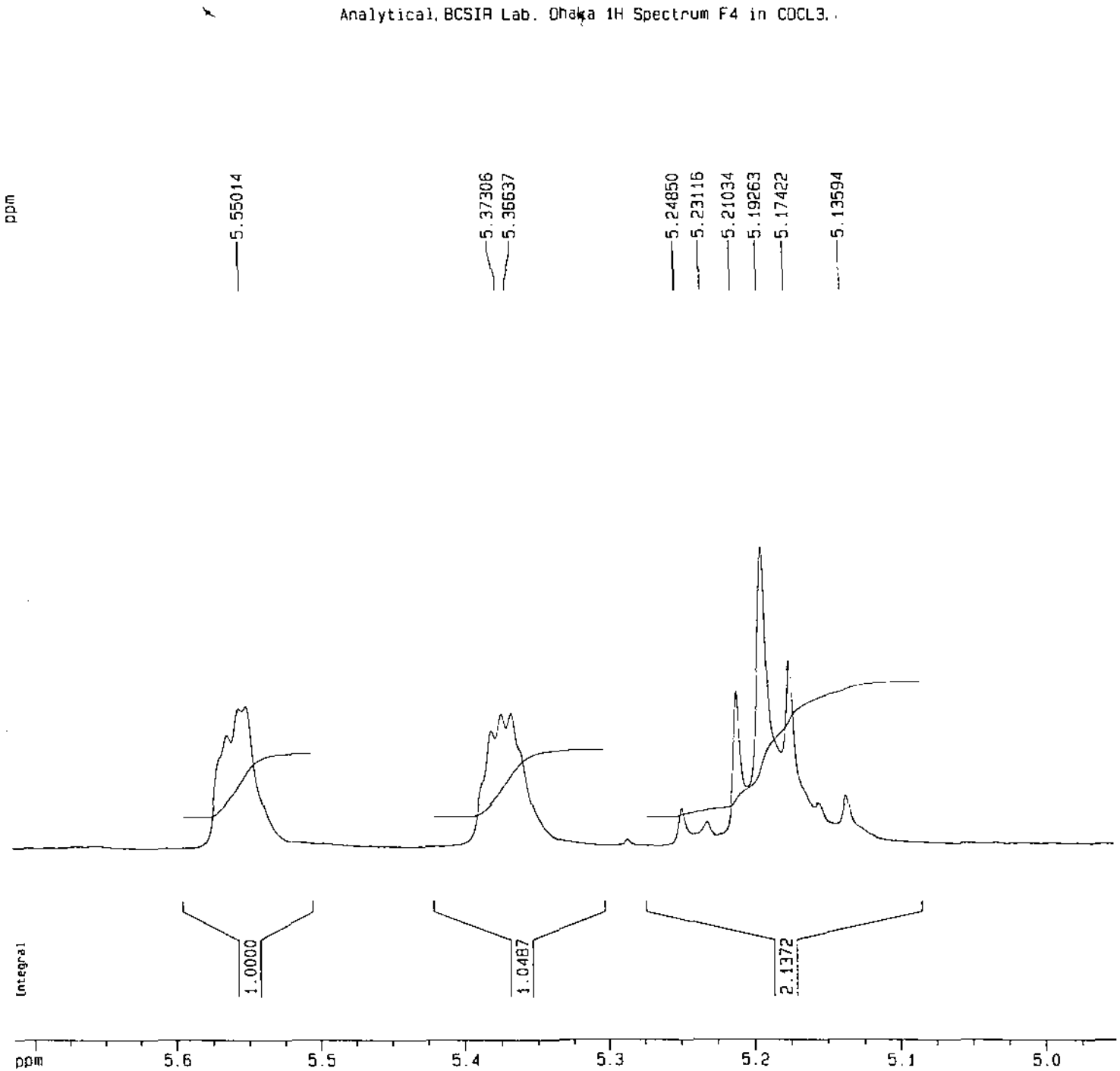



FIG 5.1.6 EXPANDED $^1$H-NMR SPECTRUM OF COMPOUND F4

Current Data Parameters

| | |
|---|---|
| NAME | A4599 |
| EXPNO | 1 |
| PROCNO | 1 |

F2 - Acquisition Parameters

| | |
|---|---|
| Date_ | 20080903 |
| Time | 12.09 |
| INSTRUM | dpx400 |
| PROBHD | 5 mm Multinuc |
| PULPROG | zg30 |
| TD | 32768 |
| SOLVENT | CDCl3 |
| NS | 128 |
| DS | 2 |
| SWH | 6410.256 Hz |
| FIDRES | 0.195625 Hz |
| AQ | 2.5559540 sec |
| RG | 181 |
| DW | 78.000 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 | 400.1428010 MHz |

F2 - Processing parameters

| | |
|---|---|
| SI | 32768 |
| SF | 400.1400126 MHz |
| WDW | EM |
| SSB | 0 |
| LB | 0.30 Hz |
| GB | 0 |
| PC | 1.40 |

1D NMR plot parameters

| | |
|---|---|
| CX | 20.00 cm |
| F1P | 5.715 ppm |
| F1 | 2286.65 Hz |
| F2P | 4.952 ppm |
| F2 | 1981.42 Hz |
| PPMCM | 0.03814 ppm/cm |
| HZCM | 15.26160 Hz/cm |

### 5.1.4 $^{13}$C-NMR SPECTROSCOPIC ANALYSIS OF COMPOUND F4

The $^{13}$C NMR spectrum (Fig.5.1.7-5.1.10) revealed the presence of 28 carbons for a steroid skeleton. The signals at δ 139.816 (C-5), δ 141.369 (C-8), 37.069 (C-10) and δ 42.859 (C-13) ppm were assigned to four quaternary carbons respectively.

The signal at δ 71.8 ppm was assigned for methylene carbon C-3. The signal at δ 138.4 ppm and δ 129.4 ppm was for the C=C carbons of C-22 and C-23 and also the signal at δ 141.0 ppm and δ 121.7 ppm was typical for the C=C carbons of C-5 and C-6.

**Table-5.2: $^{13}C$ NMR spectral data of the isolated compound F4.**

| No. of carbon | Types of Carbon | Value of δ | |
|---|---|---|---|
| | | Observed value δ | Reporte δvalue[115] |
| 1 | $=CH_2$ | 38.415 | 38.4 |
| 2 | $=CH_2$ | 32.042 | 32.0 |
| 3 | =CH- | 70.496 | 70.5 |
| 4 | $=CH_2$ | 40.843 | 40.8 |
| 5 | =C= | 139.816 | 139.8 |
| 6 | =CH- | 119.623 | 119.6 |
| 7 | =CH- | 116.328 | 116.3 |
| 8 | =C= | 141.369 | 141.4 |
| 9 | -CH- | 46.304 | 46.3 |
| 10 | =C= | 37.069 | 37.1 |
| 11 | $=CH_2$ | 21.128 | 21.2 |
| 12 | $=CH_2$ | 39.1326 | 39.1 |
| 13 | =C= | 42.859 | 42.9 |
| 14 | =CH- | | |
| 15 | $=CH_2$ | 23.024 | 23.0 |
| 16 | $=CH_2$ | 28.295 | 28.3 |
| 17 | -CH- | 55.798 | 55.8 |
| 18 | $-CH_3$ | 12.073 | 12.1 |
| 19 | $-CH_3$ | 16.310 | 16.3 |
| 20 | -CH- | 40.415 | 40.4 |
| 21 | $-CH_3$ | 21.128 | 21.1 |
| 22 | =CH- | 135.596 | 135.6 |
| 23 | =CH- | 132.028 | 132 |
| 24 | =CH- | 42.859 | 42.9 |
| 25 | =CH- | 33.122 | 33.1 |
| 26 | $-CH_3$ | 19.965 | 20.0 |
| 27 | $-CH_3$ | 19.664 | 19.7 |
| 28 | $-CH_3$ | 17.620 | 17.6 |

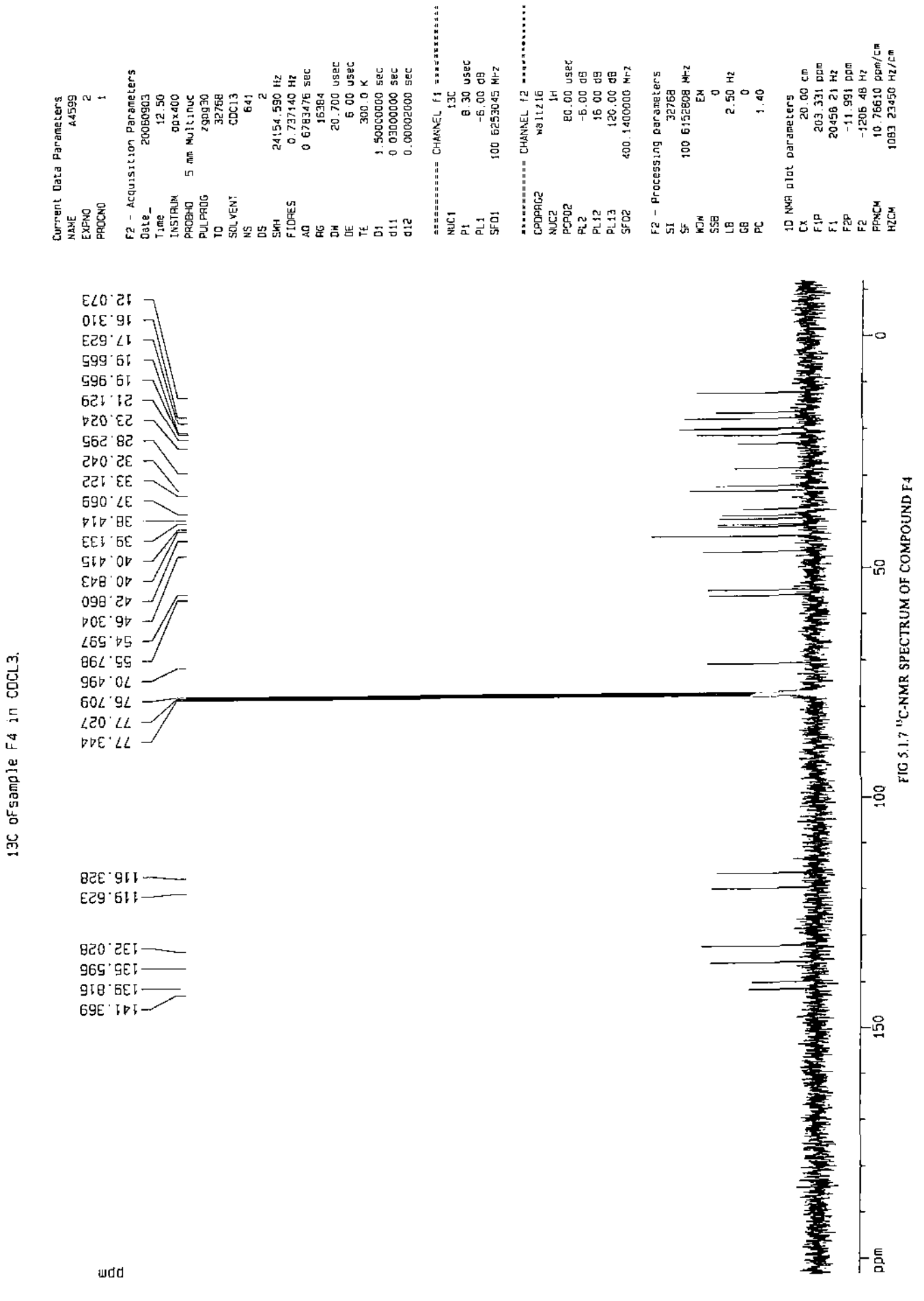



FIG 5.1.7 $^{13}$C-NMR SPECTRUM OF COMPOUND F4

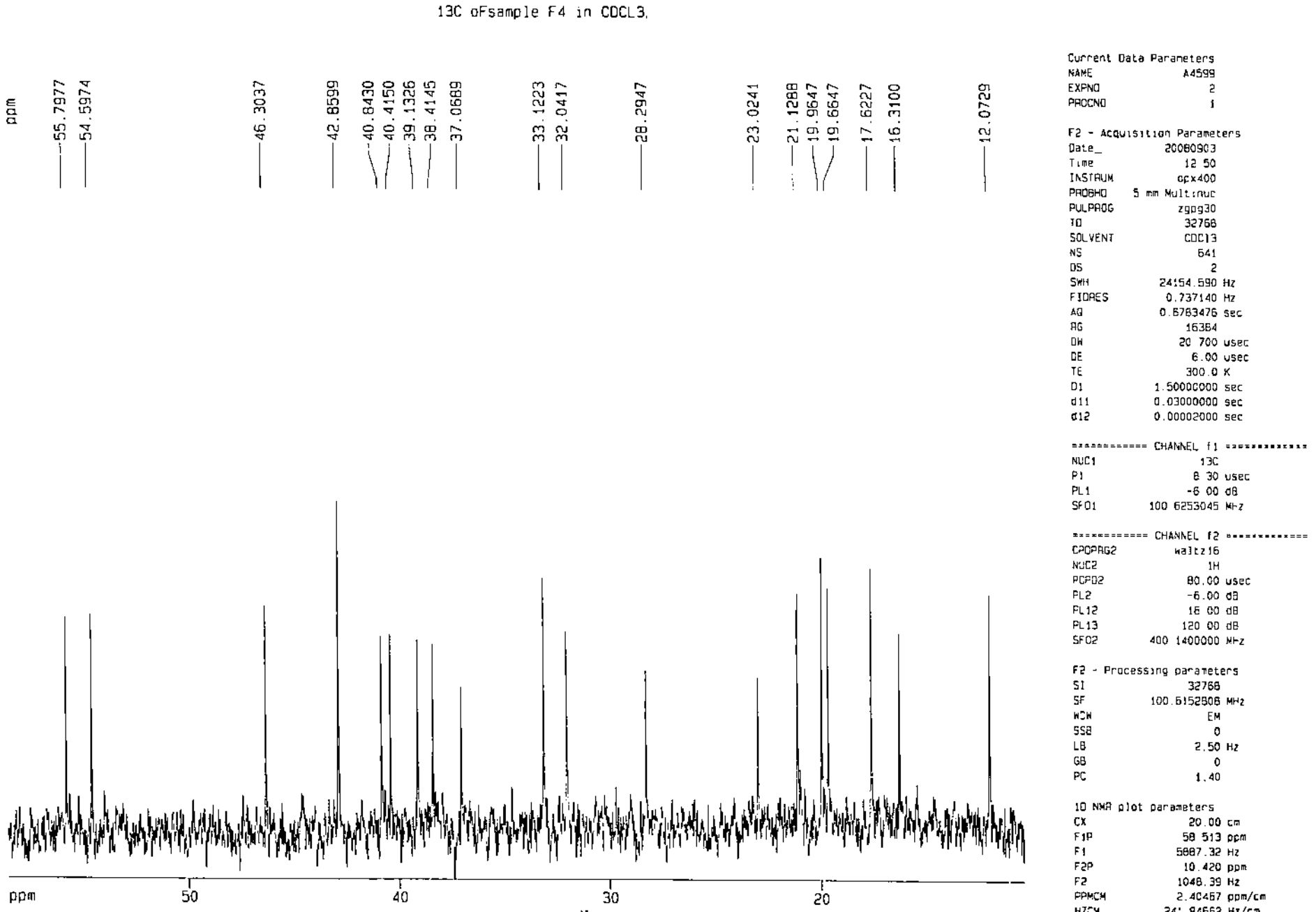



FIG 5.1.8 EXPANDED $^{13}$C-NMR SPECTRUM OF COMPOUND F4

```
Current Data Parameters
NAME                  A4599
EXPNO                     2
PROCNO                    1

F2 - Acquisition Parameters
Date_              20080903
Time                  12 50
INSTRUM             qcx400
PROBHD     5 mm Multinuc
PULPROG             zgpg30
TD                    32768
SOLVENT               CDCl3
NS                      641
DS                        2
SWH               24154.590 Hz
FIDRES             0.737140 Hz
AQ                 0.6783476 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            100.6253045 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              100.6152808 MHz
WDW                     EM
SSB                      0
LB                    2.50 Hz
GB                       0
PC                    1.40

1D NMR plot parameters
CX                   20.00 cm
F1P                 58.513 ppm
F1                5887.32 Hz
F2P                 10.420 ppm
F2                1048.39 Hz
PPMCM              2.40467 ppm/cm
HZCM              241.94662 Hz/cm
```

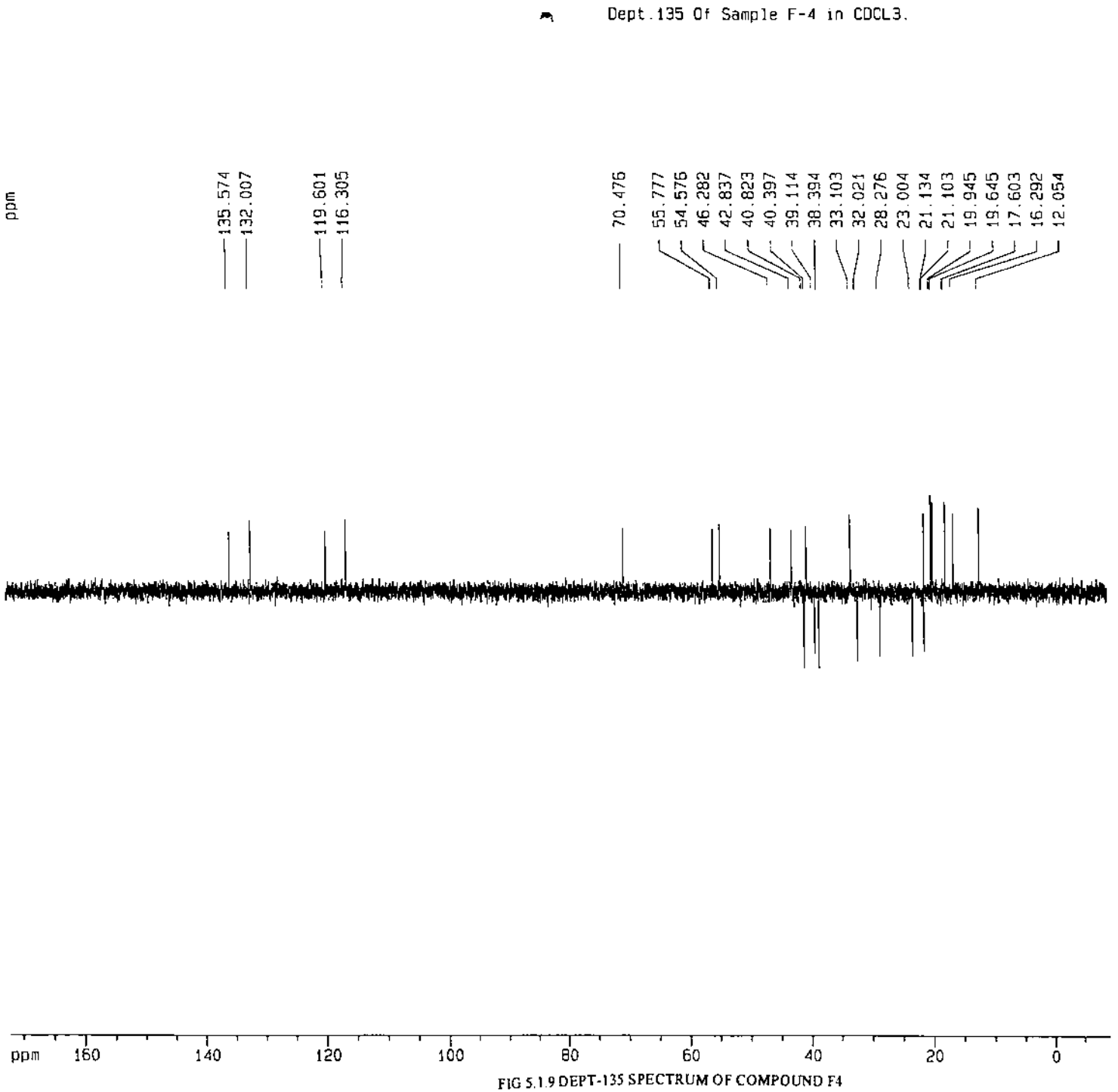



FIG 5.1.9 DEPT-135 SPECTRUM OF COMPOUND F4

Current Data Parameters

| | |
|---|---|
| NAME | A4599 |
| EXPNO | 3 |
| PROCNO | 1 |

F2 - Acquisition Parameters

| | |
|---|---|
| Date_ | 20080903 |
| Time | 13 17 |
| INSTRUM | dpx400 |
| PROBHD | 5 mm Multinuc |
| PULPROG | dept135 |
| TD | 32768 |
| SOLVENT | CDCl3 |
| NS | 116 |
| DS | 8 |
| SWH | 24154.590 Hz |
| FIDRES | 0.737140 Hz |
| AQ | 0.6783476 sec |
| RG | 13004 |
| DW | 20.700 usec |
| DE | 6.00 usec |
| TE | 300.0 K |
| CNST2 | 145.0000000 |
| D1 | 4.00000000 sec |
| d2 | 0.00344828 sec |
| d12 | 0.00002000 sec |
| DELTA | 0.00000764 sec |

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

| | |
|---|---|
| NUC1 | 13C |
| P1 | 6.00 usec |
| p2 | 12.00 usec |
| PL1 | -6.00 dB |
| SFO1 | 100.6253045 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 | 100.6152828 MHz |
| WDW | EM |
| SSB | 0 |
| LB | 1.00 Hz |
| GB | 0 |
| PC | 1.40 |

1D NMR plot parameters

| | |
|---|---|
| CX | 20.00 cm |
| F1P | 172.186 ppm |
| F1 | 17324.54 Hz |
| F2P | -9.205 ppm |
| F2 | -926.16 Hz |
| PPMCM | 9.08955 ppm/cm |
| HZCM | 912.53529 Hz/cm |

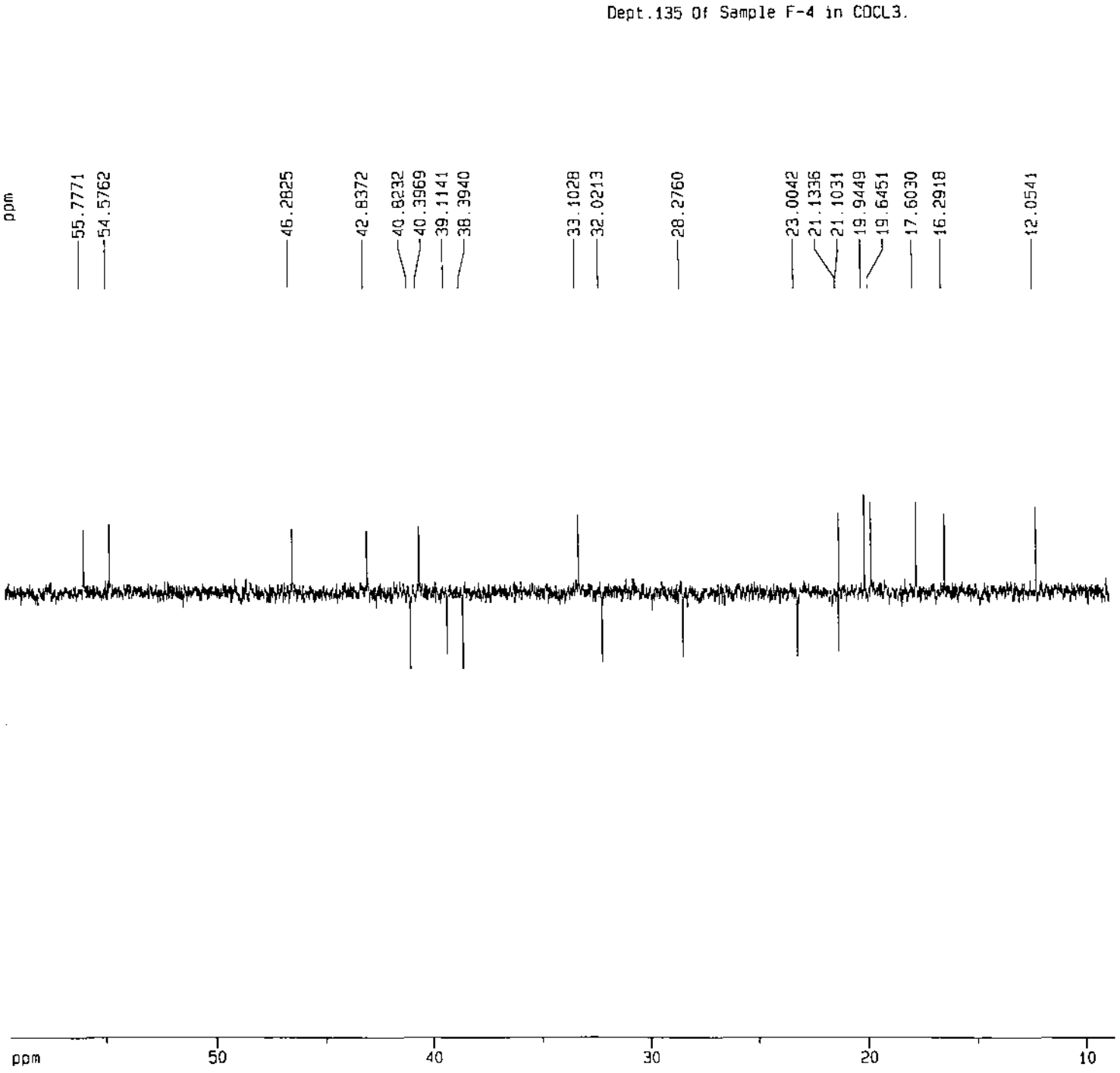



FIG 5.1.10 EXPANDED DEPT-135 SPECTRUM OF COMPOUND F4

Current Data Parameters

| | |
|---|---|
| NAME | A4599 |
| EXPNO | 3 |
| PROCNO | 1 |

F2 - Acquisition Parameters

| | |
|---|---|
| Date_ | 20080903 |
| Time | 13.17 |
| INSTRUM | dpx400 |
| PROBHD | 5 mm Multinuc |
| PULPROG | dept135 |
| TD | 32768 |
| SOLVENT | CDCl3 |
| NS | 116 |
| DS | 8 |
| SWH | 24154.590 Hz |
| FIDRES | 0.737140 Hz |
| AQ | 0.6783476 sec |
| RG | 13004 |
| DW | 20.700 usec |
| DE | 6.00 usec |
| TE | 300.0 K |
| CNST2 | 145.0000000 |
| D1 | 4.00000000 sec |
| d2 | 0.00344828 sec |
| d12 | 0.00002000 sec |
| DELTA | 0.00000764 sec |

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

| | |
|---|---|
| NUC1 | 13C |
| P1 | 6.00 usec |
| p2 | 12.00 usec |
| PL1 | -6.00 dB |
| SFO1 | 100.6253045 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 |

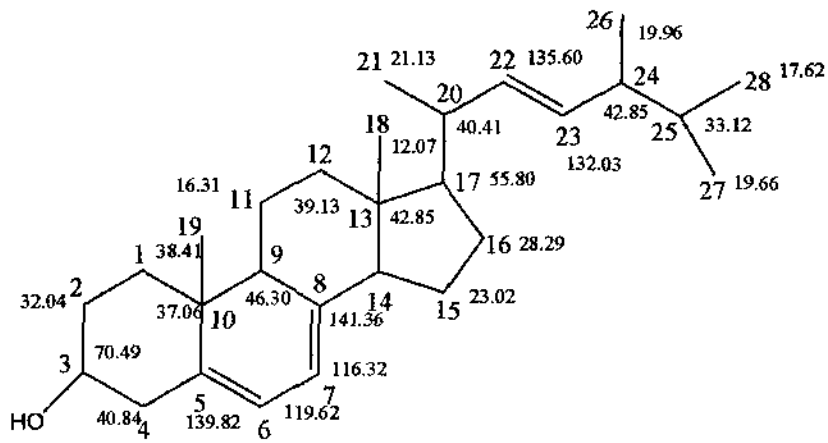
F2 - Processing parameters

| | |
|---|---|
| SI | 32768 |
| SF | 100.6152828 MHz |
| WDW | EM |
| SSB | 0 |
| LB | 1.00 Hz |
| GB | 0 |
| PC | 1.40 |

1D NMR plot parameters

| | |
|---|---|
| CX | 20.00 cm |
| F1P | 59.419 ppm |
| F1 | 5978.46 Hz |
| F2P | 8.722 ppm |
| F2 | 877.57 Hz |
| PPMCM | 2.53485 ppm/cm |
| HZCM | 255.04440 Hz/cm |

Thus the structure of compound F4 has been identified as following



File :D:\MSDCHEM\1\DATA\TRAINING\QADER\Snapshot\TAC-a.D
Operator : Dr. Soko
Acquired : 17 Mar 2008 15:57 using AcqMethod MGA1.M
Instrument : JAS GC-MS
Sample Name: TAC
Misc Info : Tofail
Vial Number: 21



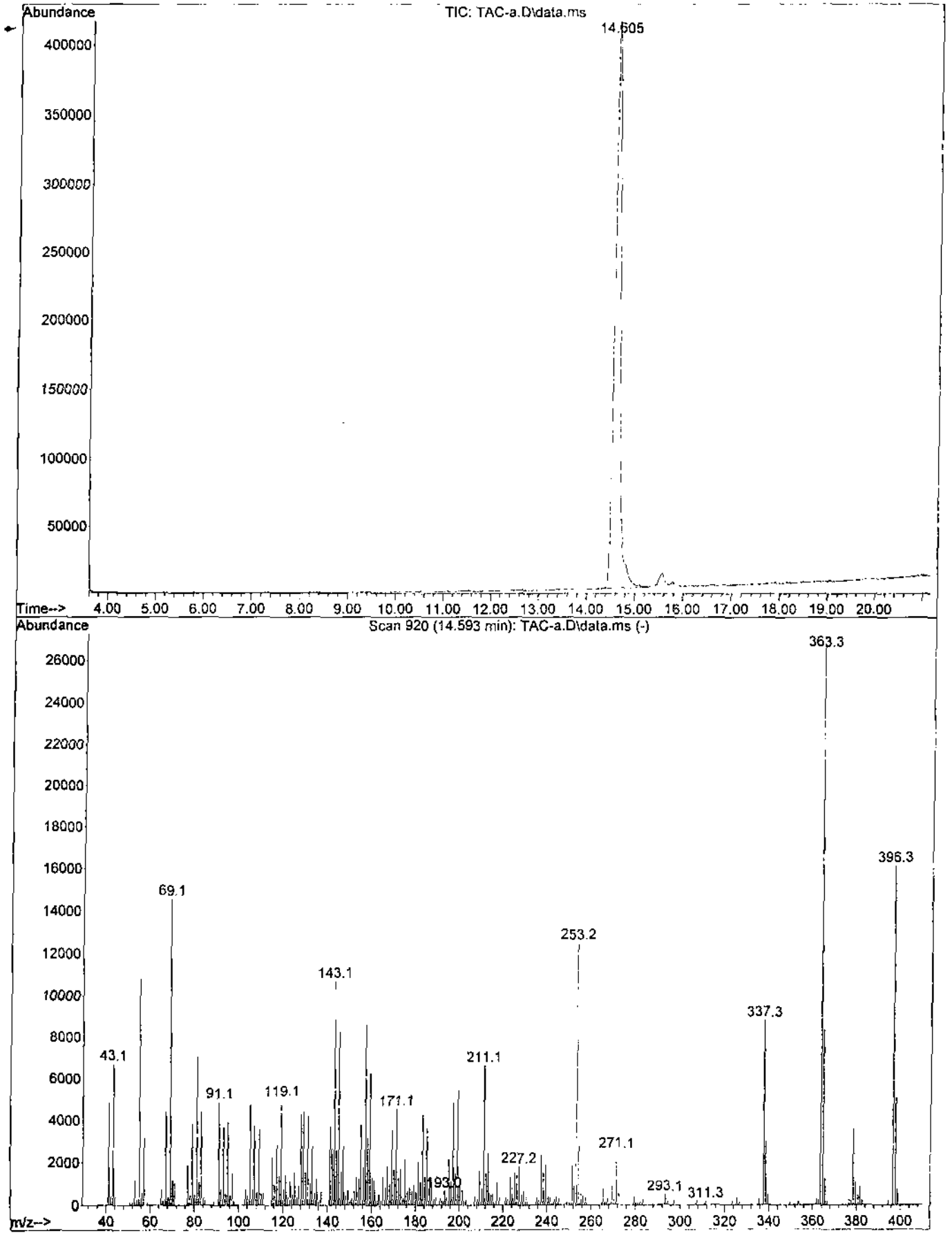



FIG 5.1.11 GC-MS SPECTRUM OF COMPOUND F4

## 5.1.5 MASS SPECTROSCOPIC ANALALYSIS OF COMPOUND F4

The compound has a molecular weight of 396, according to the mass spectrum and agreeing with condenced formula $C_{28}H_{44}O$. The fragmentation pattern for this molecule is typical of sterols with ions at m/z 381(M+-CH3), 378(M+-H2O), 363(M+-methyl-H2O), 271 (M+-side chain), 253(M+-side chain-H2O), 211(fission of D ring-H2O),143 (M+-H2O-fission-C ring-methyl), 337 (M+-59) and 157 being the last two typical for the location of an unsaturation in C-22 of the side chain; agreeing completely with the fragmentation reported in literature for the compound Ergosta-5,7,22-trien-b-ol(Ergosterol), commonly distributed inside fungi kingdom. The structure of F4 has been finally established by the combined 1H, 13C NMR DEPT and mass, spectral data. All these spectral data were in good agreement with the reported spectral data of Ergosterol. Thus the structure of the compound F4 has been identified as Ergosterol.

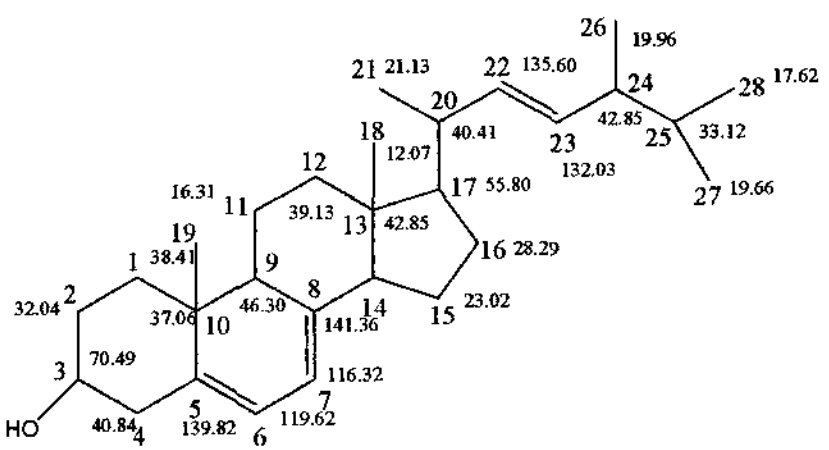



Fig 5.1.2: Structure of Ergesterol

## 5.2 CHARACTERISATION OF COMPOUND B3

The compound B3 (75.2 mg) was isolated as reddish color substance from the dichloromethane extract of the plant *Pleurotus Ostreatus* upon vacuum liquid chromatography and crystallization of the fraction $F_6$. It was soluble in dichloromethane . Its TLC study indicated the presence of a single spot ($R_f$= 0.66 DCM:MeOH=95:05 in over silica gel GF254, dichloromethane: methanol =95:05 as the mobile phase). The spot was visualized as a violet colour upon spraying with vanillin-sulfuric acid followed by heating in an oven at 110°C.

### 5.2.1 IR SPECTROSCOPIC ANALYSIS OF COMPOUND B3

The IR[113] spectrum (Fig-5.2.1) of the compound B3 showed an absorption band at (3600-3500) $cm^{-1}$ indicating the presence of -OH group. The band at 2987 $cm^{-1}$ was for C-H stretching and 1363 $cm^{-1}$ for -CH3 group. The peak at 1637 $cm^{-1}$ was suggestive the presence of >C=C< group. The band at 1421 $cm^{-1}$ indicated $-CH_2$-group. The absorption band at 1710 $cm^{-1}$ was suggestive of C=O stretching. The other peak at 720 $cm^{-1}$ indicated the presence of long chain band.

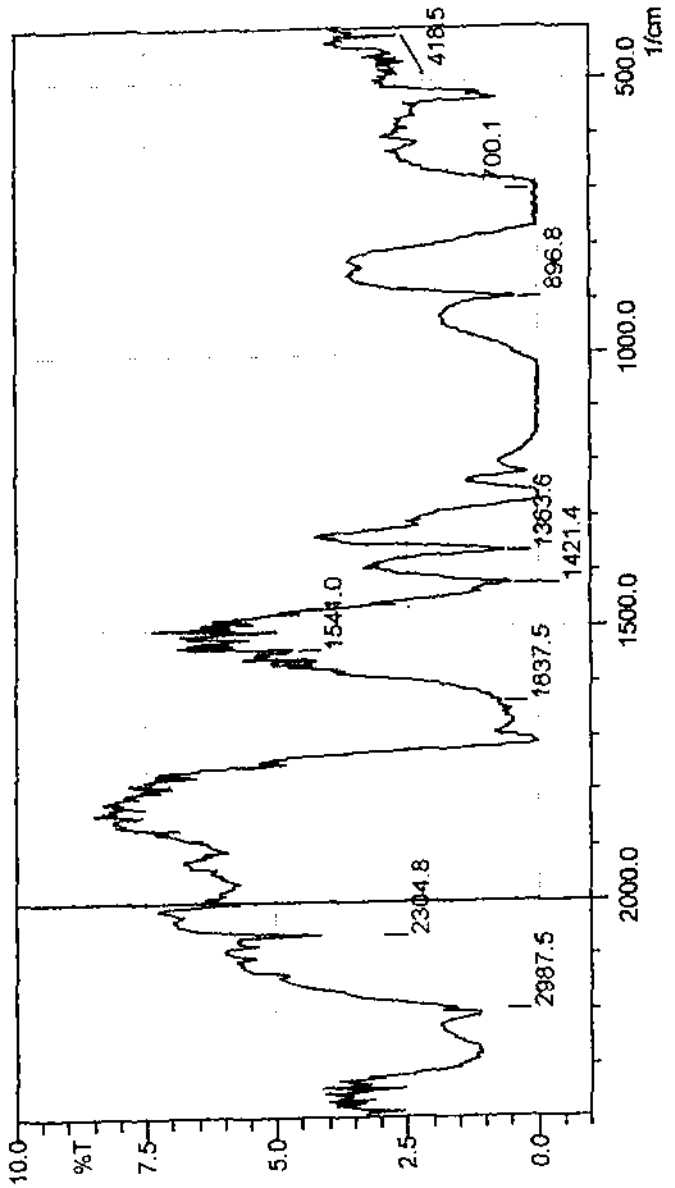



FIG 5.2.1 IR SPECTRUM OF COMPOUND B3

## 5.2.2 $^1$H-NMR SPECTROSCOPIC ANALYSIS OF COMPOUND B3

The $^1$H-NMR (400MHz in $CDCl_3$) spectrum (Fig-5.2.2-5.2.6) of the isolated compound B3 showed a downfield doublet doublet at chemilal shift, δ 7.420 and 7.250 ppm suggestive of the presence of olefmic proton (2-H and 3-H).

The $^1$H-NMR spectrum displayed doublet-doublet at δ 5.685 ppm for the proton at C-4. The spectrum showed a broad banded multiple at δ 4.068- 4.141 ppm suggestive of an oxymethine proton 5-H.

The proton of acetoxy group at C-4 showed a singlet at δ 2.034 ppm. Methelene proton at C-8 showed multiple at δ 1.620 ppm and methyl proton at C-20 showed triplet at δ 0.876 ppm. The chemilal shift, δ 1.245-1.320 ppm showed multiphe for the methelene proton at C-9 and C-19. The chemilal shift, δ 2.758 ppm and δ 2.308 ppm for H-7A and H-7B.

**Table-5.3: $^1$H-NMR spectral data of compound B3**

| No.of protons | Chemical shift value(δ) | |
|---|---|---|
| | Experiment value(δ) | Reported value[116] (δ) |
| H-3 | 7.420 | 7.700(dd, J=6.2/2.1 Hz) |
| H-2 | 7.250 | 6.591( dd, J=6.2/1.7) |
| H-4 | 5.685 | 5.826(dd, J=2.1/1.7) |
| 5-OH | 4.141 | 4.613(s) |
| H-7A | 2.758 | 2.590(ddd17.9/7.2/7.1) |
| H-7B | 2.308 | 2.319(ddd,J=17.9/7.5/6.7Hz) |
| 4-OAc | 2.034 | 2.088(s) |
| H-8 | 1.620 | 1.619(m) |
| H-9-H-19 | 1.245-1.320 | 1.20-1.33(m) |
| H-20 | 0.876 | 0.878 (t) |

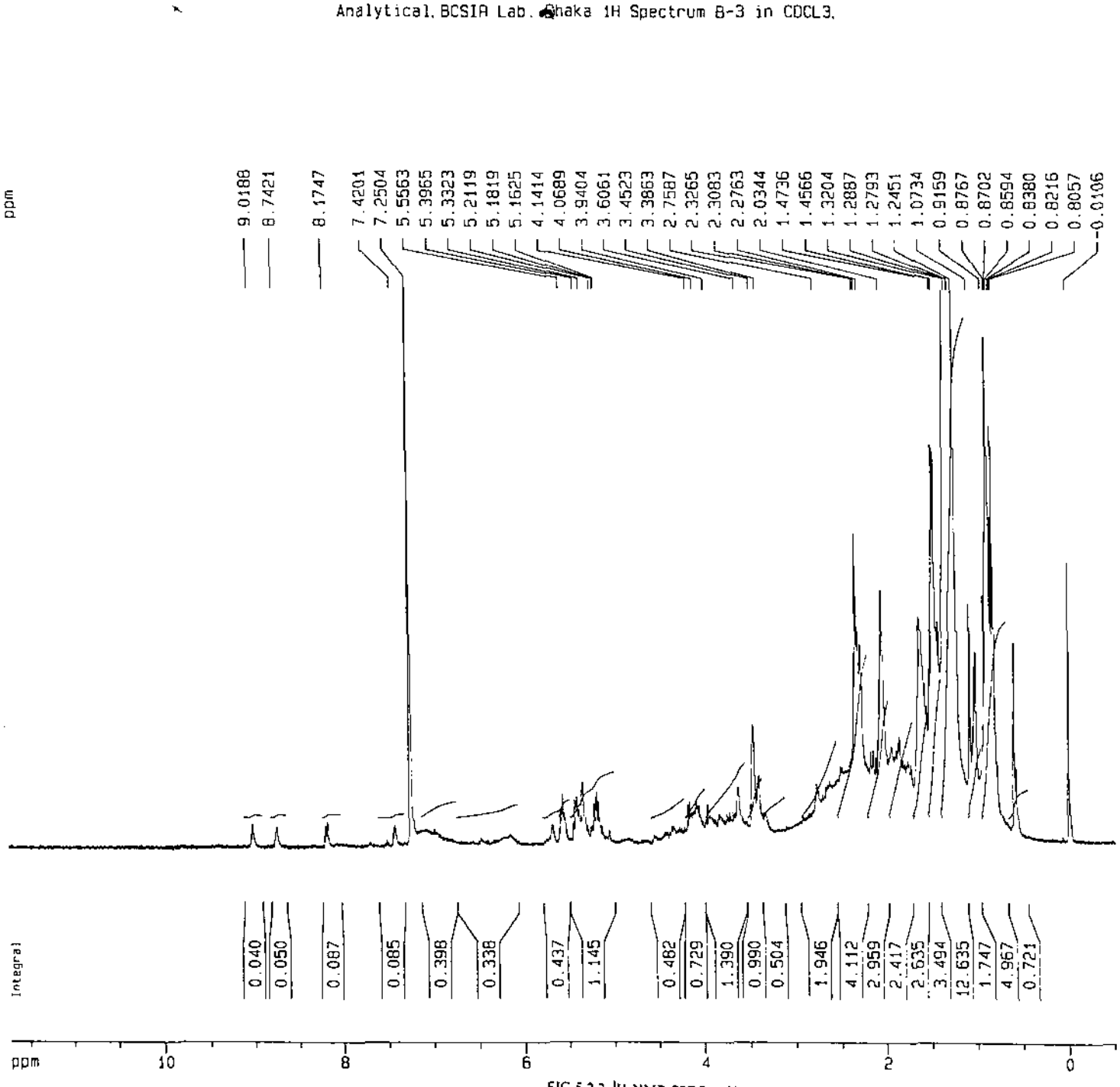



FIG 5.2.2 $^{1}$H-NMR SPECTRUM OF COMPOUND B3

Current Data Parameters

| | |
|---|---|
| NAME | A4598 |
| EXPNO | 1 |
| PROCNO | 1 |

F2 - Acquisition Parameters

| | |
|---|---|
| Date_ | 20080903 |
| Time | 11.56 |
| INSTRUM | dpx400 |
| PROBHD | 5 mm Multinuc |
| PULPROG | zg30 |
| TD | 32768 |
| SOLVENT | CDCl3 |
| NS | 128 |
| DS | 2 |
| SWH | 6410.256 Hz |
| FIDRES | 0.195625 Hz |
| AQ | 2.5559540 sec |
| RG | 362 |
| DW | 78.000 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 | 400.1428010 MHz |

F2 - Processing parameters

| | |
|---|---|
| SI | 32768 |
| SF | 400.1400124 MHz |
| WDW | EM |
| SSB | 0 |
| LB | 0.30 Hz |
| GB | 0 |
| PC | 1.40 |

1D NMR plot parameters

| | |
|---|---|
| CX | 20.00 cm |
| F1P | 11.727 ppm |
| F1 | 4692.53 Hz |
| F2P | -0.513 ppm |
| F2 | -205.42 Hz |
| PPMCM | 0.61203 ppm/cm |
| HZCM | 244.89769 Hz/cm |

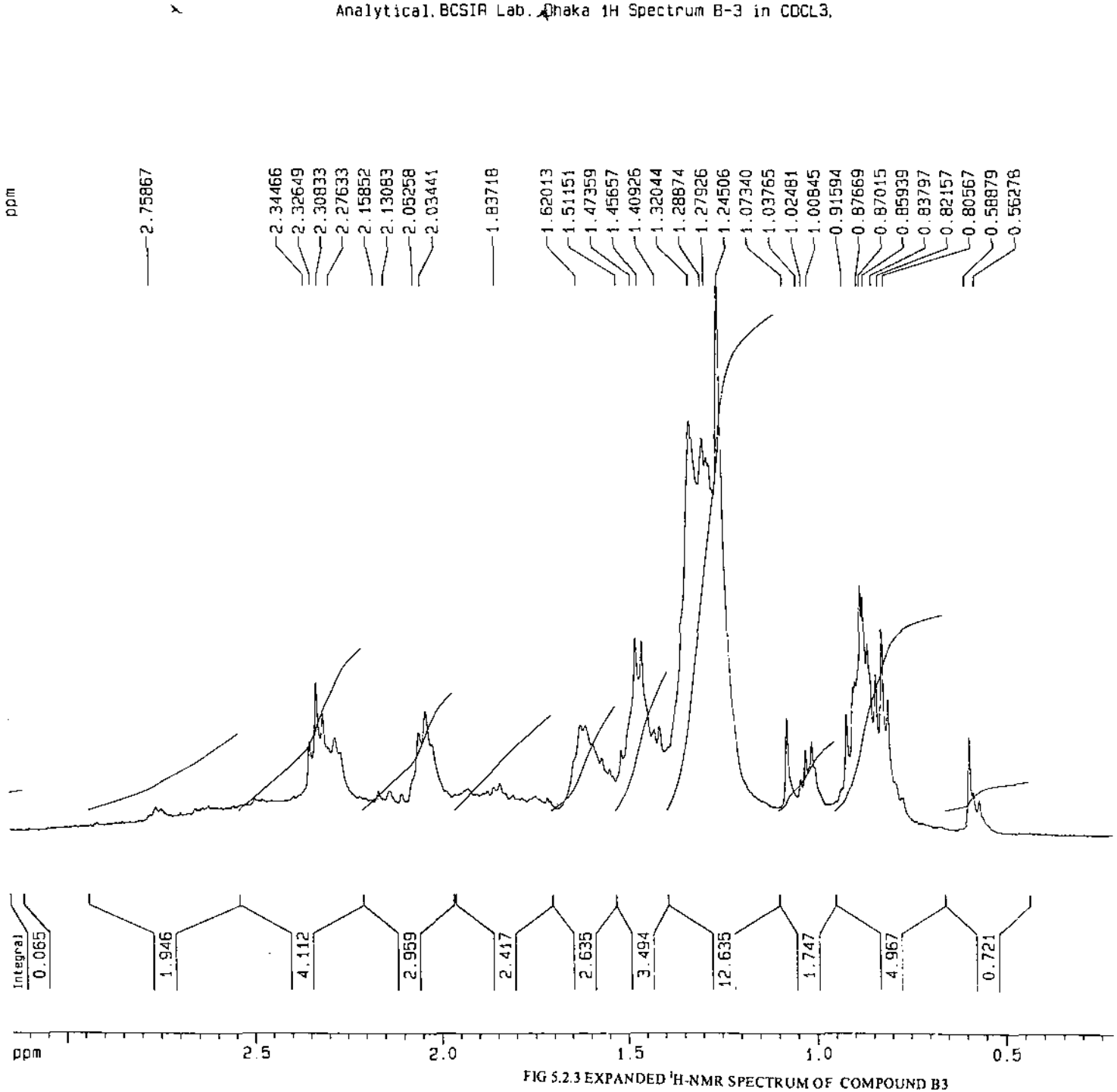



FIG 5.2.3 EXPANDED $^{1}$H-NMR SPECTRUM OF COMPOUND B3

Current Data Parameters

| | |
|---|---|
| NAME | A4598 |
| EXPNO | 1 |
| PROCNO | 1 |

F2 - Acquisition Parameters

| | |
|---|---|
| Date_ | 20080903 |
| Time | 11.56 |
| INSTRUM | dpx400 |
| PROBHD | 5 mm Multinuc |
| PULPROG | zg30 |
| TD | 32768 |
| SOLVENT | CDCl3 |
| NS | 128 |
| DS | 2 |
| SWH | 6410.256 Hz |
| FIDRES | 0.195625 Hz |
| AQ | 2.5559540 sec |
| RG | 362 |
| DW | 78.000 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 | 400.1428010 MHz |

F2 - Processing parameters

| | |
|---|---|
| SI | 32768 |
| SF | 400.1400124 MHz |
| WDW | EM |
| SSB | 0 |
| LB | 0.30 Hz |
| GB | 0 |
| PC | 1.40 |

1D NMR plot parameters

| | |
|---|---|
| CX | 20.00 cm |
| F1P | 3.147 ppm |
| F1 | 1259.20 Hz |
| F2P | 0.219 ppm |
| F2 | 87.50 Hz |
| PPMCM | 0.14641 ppm/cm |
| HZCM | 58.58471 Hz/cm |

Analytical, BCSIR Lab. Dhaka 1H Spectrum B-3 in CDCL3.

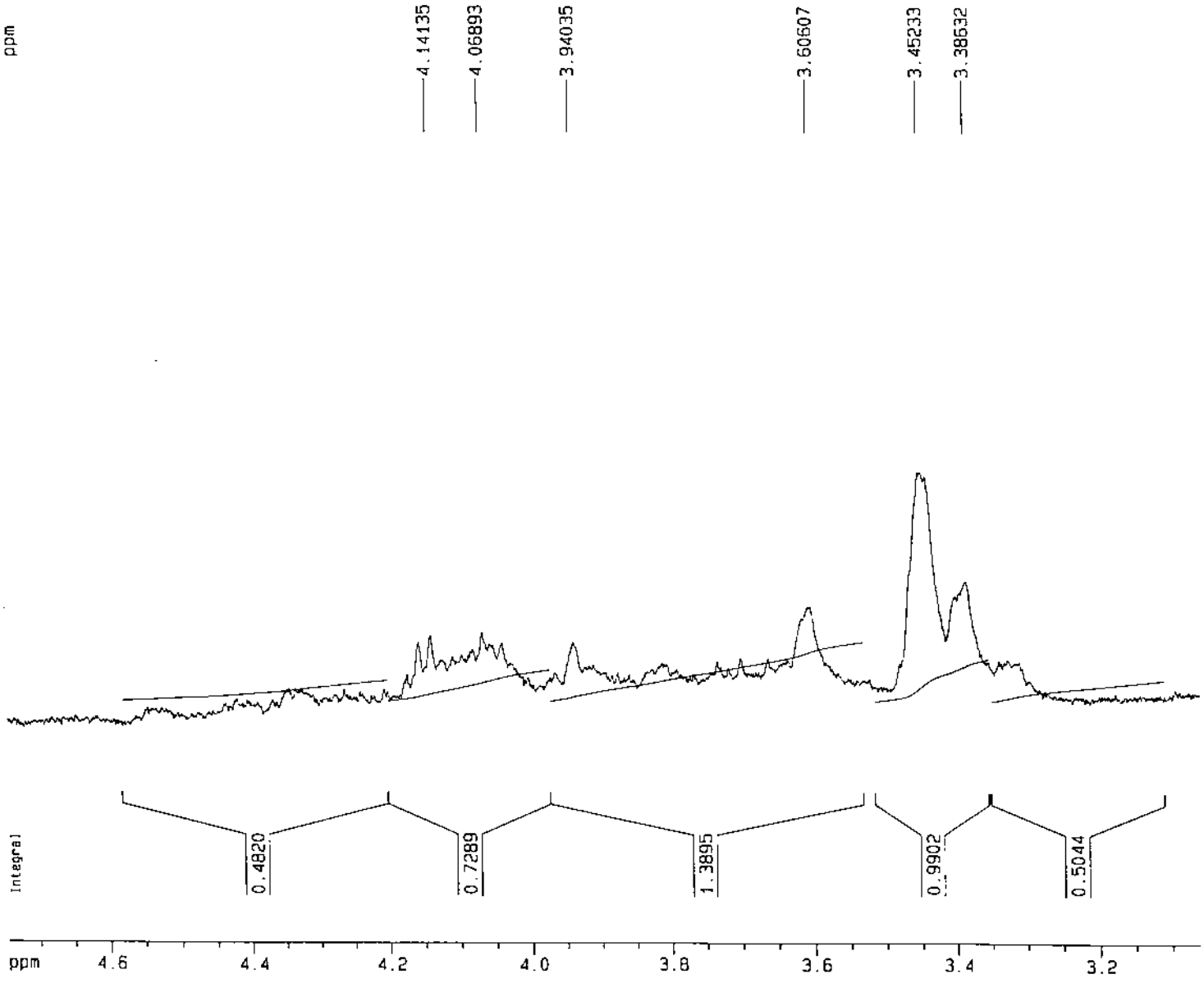



FIG 5.2.4 EXPANDED $^{1}$H-NMR SPECTRUM OF COMPOUND B3

Current Data Parameters

| | |
|---|---|
| NAME | A4598 |
| EXPNO | 1 |
| PROCNO | 1 |

F2 - Acquisition Parameters

| | |
|---|---|
| Date_ | 20080903 |
| Time | 11.56 |
| INSTRUM | dpx400 |
| PROBHD | 5 mm Multinuc |
| PULPROG | zg30 |
| TD | 32768 |
| SOLVENT | CDCl3 |
| NS | 128 |
| DS | 2 |
| SWH | 6410.256 Hz |
| FIDRES | 0.195625 Hz |
| AQ | 2.5559540 sec |
| RG | 362 |
| DW | 78.000 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 | 400.1428010 MHz |

F2 - Processing parameters

| | |
|---|---|
| SI | 32768 |
| SF | 400.1400124 MHz |
| WDW | EM |
| SSB | 0 |
| LB | 0.30 Hz |
| GB | 0 |
| PC | 1.40 |

1D NMR plot parameters

| | |
|---|---|
| CX | 20.00 cm |
| F1P | 4.747 ppm |
| F1 | 1899.54 Hz |
| F2P | 3.062 ppm |
| F2 | 1225.14 Hz |
| PPMCM | 0.08427 ppm/cm |
| HZCM | 33.72027 Hz/cm |

Analytical, BCSIR Lab., Dhaka 1H Spectrum B-3 in CDCL3.

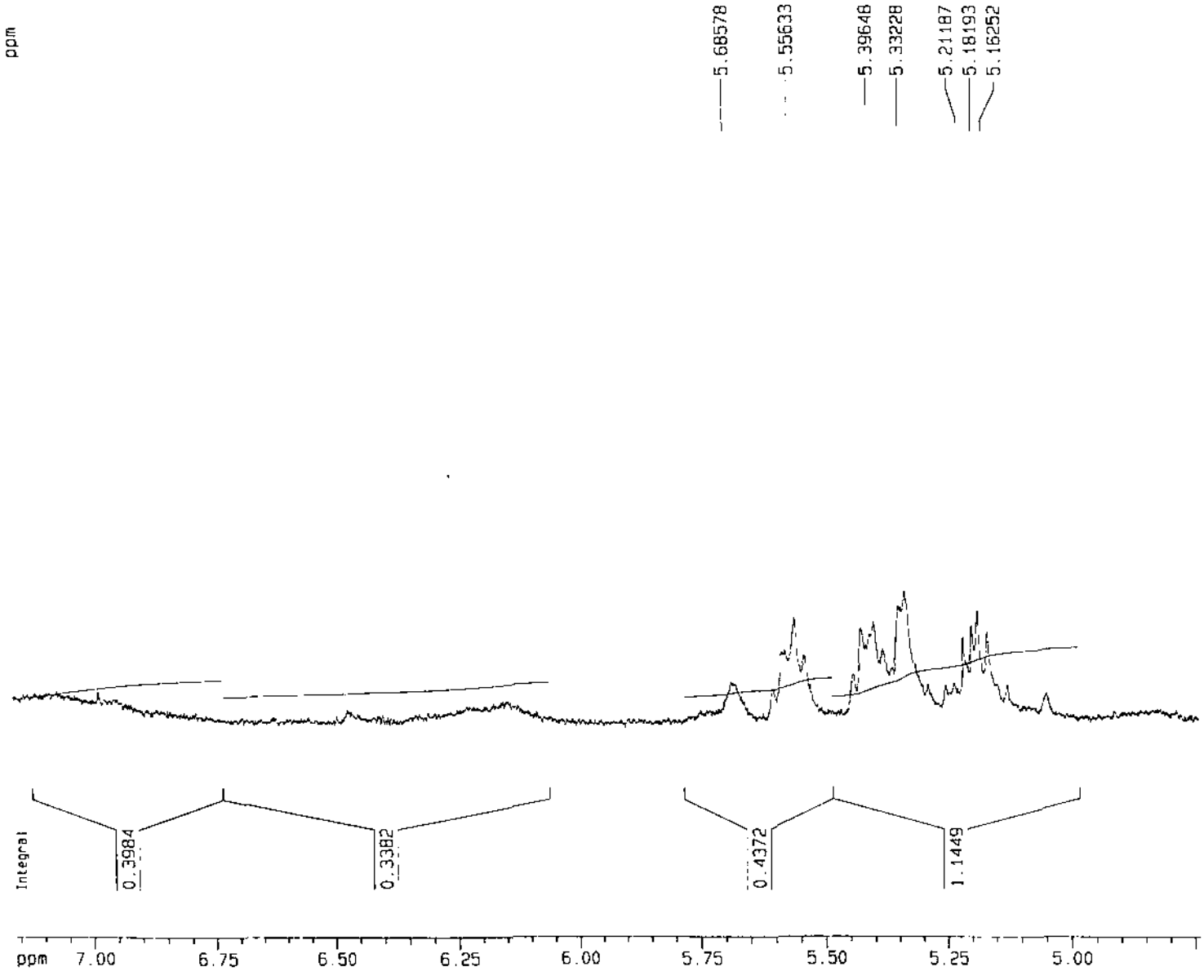



FIG 5.2.5 EXPANDED $^1$H-NMR SPECTRUM OF COMPOUND B3

Current Data Parameters

| | |
|---|---|
| NAME | A4598 |
| EXPNO | 1 |
| PROCNO | 1 |

F2 - Acquisition Parameters

| | |
|---|---|
| Date_ | 20080903 |
| Time | 11.56 |
| INSTRUM | dpx400 |
| PROBHD | 5 mm Multinuc |
| PULPROG | zg30 |
| TD | 32768 |
| SOLVENT | CDCl3 |
| NS | 128 |
| DS | 2 |
| SWH | 6410.256 Hz |
| FIDRES | 0.195625 Hz |
| AQ | 2.5559540 sec |
| RG | 362 |
| DW | 78.000 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 | 400.1428010 MHz |

F2 - Processing parameters

| | |
|---|---|
| SI | 32768 |
| SF | 400.1400124 MHz |
| WDW | EM |
| SSB | 0 |
| LB | 0.30 Hz |
| GB | 0 |
| PC | 1.40 |

1D NMR plot parameters

| | |
|---|---|
| CX | 20.00 cm |
| F1P | 7.161 ppm |
| F1 | 2865.57 Hz |
| F2P | 4.738 ppm |
| F2 | 1895.94 Hz |
| PPMCM | 0.12116 ppm/cm |
| HZCM | 48.48155 Hz/cm |

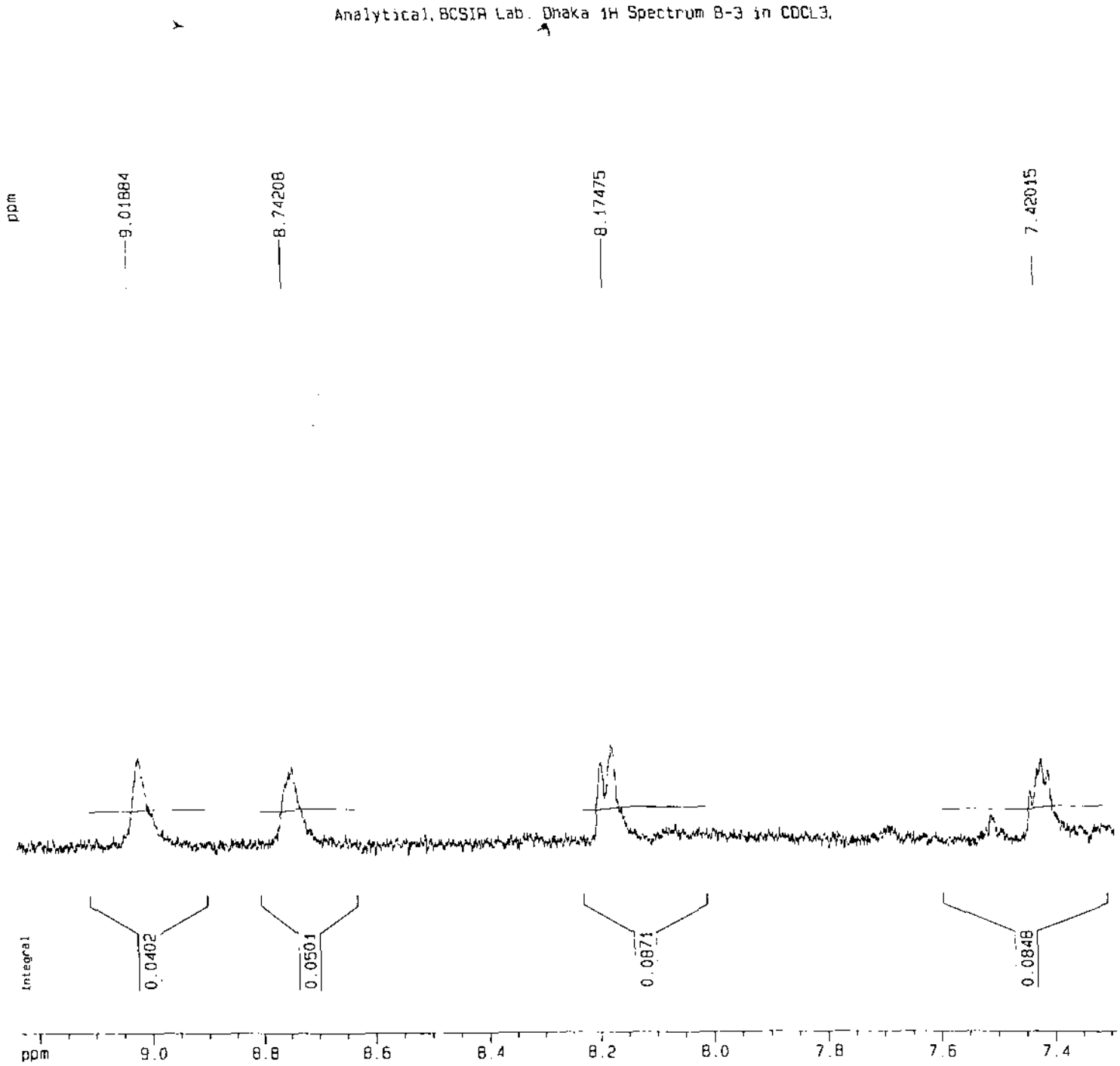



FIG 5.2.6 EXPANDED $^1$H-NMR SPECTRUM OF COMPOUND B3

Current Data Parameters

| | |
|---|---|
| NAME | A4598 |
| EXPNO | 1 |
| PROCNO | 1 |

F2 - Acquisition Parameters

| | |
|---|---|
| Date_ | 20080903 |
| Time | 11.56 |
| INSTRUM | dpx400 |
| PROBHD | 5 mm Multinuc |
| PULPROG | zg30 |
| TD | 32768 |
| SOLVENT | CDCl3 |
| NS | 128 |
| DS | 2 |
| SWH | 6410.256 Hz |
| FIDRES | 0.195625 Hz |
| AQ | 2.5559540 sec |
| RG | 362 |
| DW | 78.000 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 | 400.1428010 MHz |

F2 - Processing parameters

| | |
|---|---|
| SI | 32768 |
| SF | 400.1400124 MHz |
| WDW | EM |
| SSB | 0 |
| LB | 0.30 Hz |
| GB | 0 |
| PC | 1.40 |

1D NMR plot parameters

| | |
|---|---|
| CX | 20.00 cm |
| F1P | 9.235 ppm |
| F1 | 3695.48 Hz |
| F2P | 7.291 ppm |
| F2 | 2917.55 Hz |
| PPMCM | 0.09721 ppm/cm |
| HZCM | 38.89653 Hz/cm |

### 5.2.3 $^{13}$C-NMR SPECTROSCOPIC ANALYSIS OF COMPOUND B3

The $^{13}$C-NMR spectrum (Fig-5.2.7-5.2.10 ) of the isolated compound B3 revealed the presence of 22 carbon signals. The signal at chemical shift,δ 14.050 ppm (C-20) was assigned to a methyl carbon. The signals at chemical shift,δ 22.055 ppm (C-19), δ 22.541 ppm (C-18), δ 22.678 ppm (C-17), δ 24.661 ppm (C-16), δ 25.473 ppm (C-15), δ 27.387 ppm (C-14), δ 28.867 ppm (C-13), δ 29.037 ppm (C-12), δ 29.283 ppm (C-11), δ 29.686 ppm (C-10), δ 31.506 ppm (C-9), δ 31.704 ppm (C-8) were assigned for methylene carbons respectively. The signal at δ 124.543 ppm (C-2) and δ 133.820 ppm (C-3) were assigned for the >C=C< carbons. The signal at δ 21.109 ppm was assigned for acetoxy methyl carbon attached to C-4 and δ 33.571 ppm was assigned for $CH_2$ attached to C-7. The signal at δ 73.660 ppm was due to the presence of CH attached to C-4.

**Table 5.4: $^{13}$C-NMR spectral data of the isolated compound B3**

| No. of carbon | Types of carbon (assigned) | Chemical shift value (Observed)[116] |
|---|---|---|
| C-20 | Methyl carbon | 14.050 |
| Methyl carbon at C-4 | Acetoxy methyl carbon | 21.109 |
| C-19 | Methylene carbon | 22.055 |
| C-18 | Methylene carbon | 22.541 |
| C-17 | Methylene carbon | 22.678 |
| C-16 | Methylene carbon | 24.661 |
| C-15 | Methylene carbon | 25.473 |
| C-14 | Methylene carbon | 27.387 |
| C-13 | Methylene carbon | 28.867 |
| C-12 | Methylene carbon | 29.037 |
| C-11 | Methylene carbon | 29.283 |
| C-10 | Methylene carbon | 29.686 |
| C-9 | Methylene carbon | 31.506 |
| C-8 | Methylene carbon | 31.704 |
| C-7 | $CH_2$ | 33.571 |
| C-4 | CH | 73.660 |
| C-5 | Quartenary carbon | 77.350 |
| C-2 | Olefinic | 124.543 |
| C-3 | Olefinic | 133.820 |

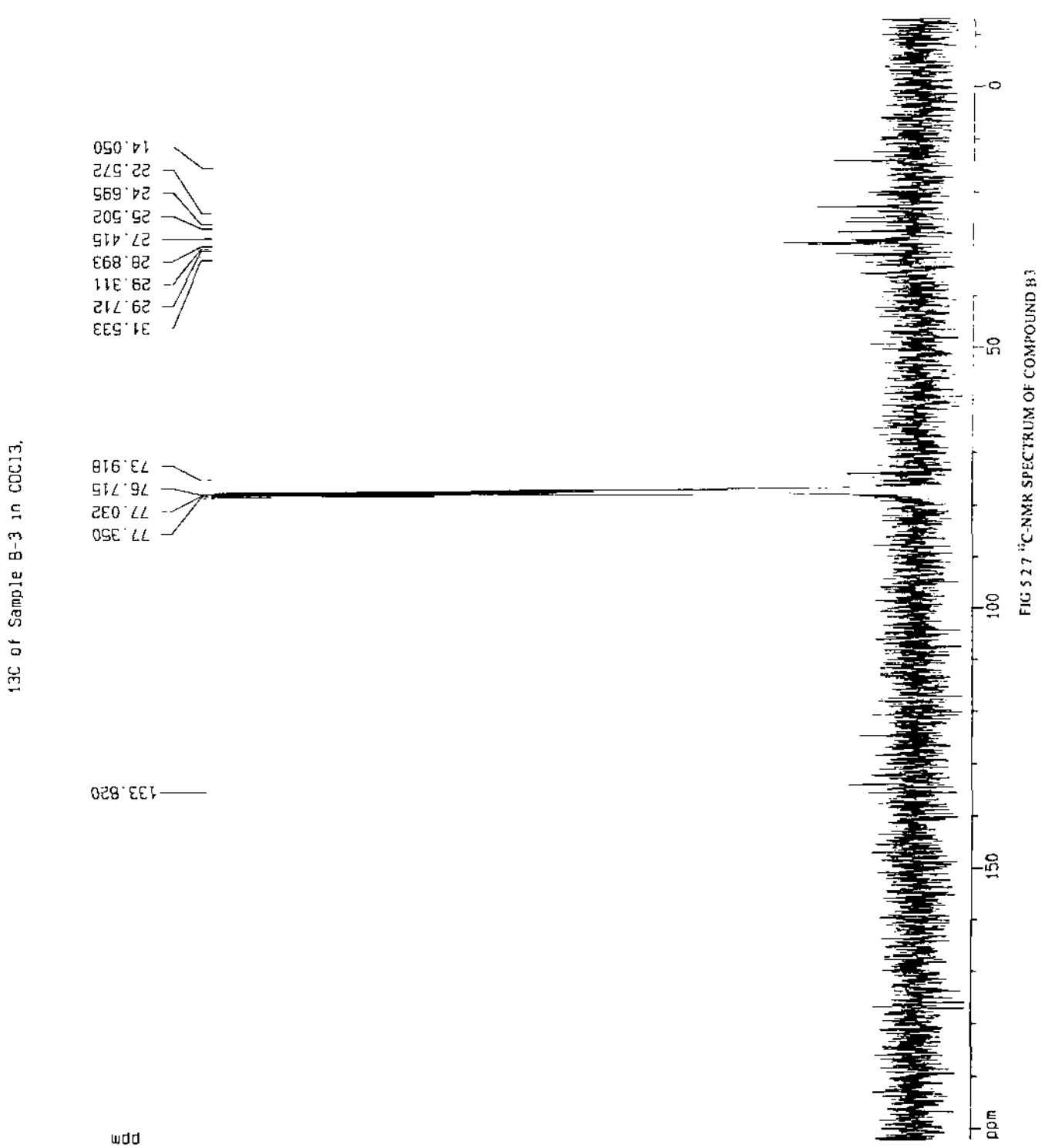



FIG 5.2.7 $^{13}$C-NMR SPECTRUM OF COMPOUND B3

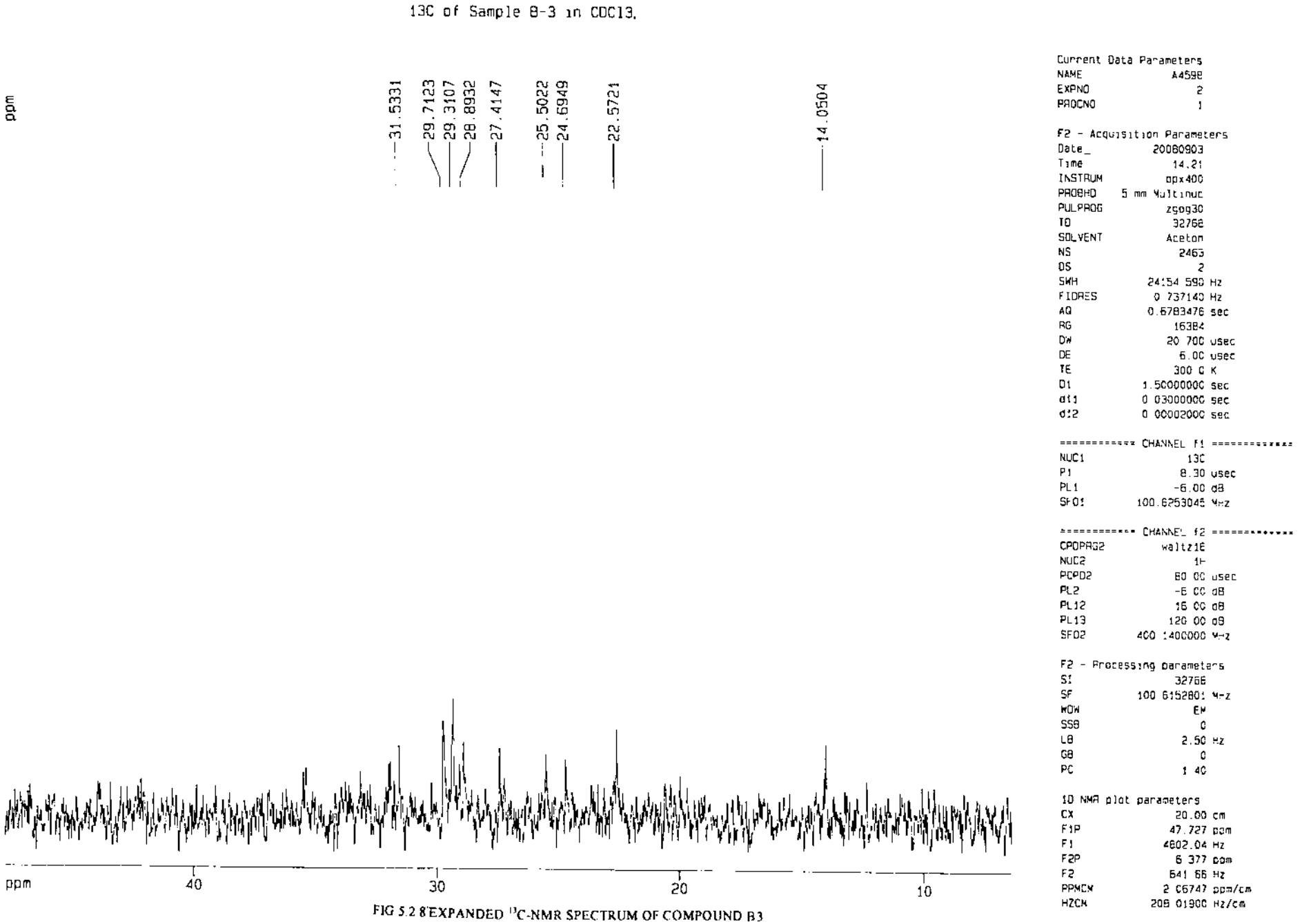



FIG 5.2.8 EXPANDED $^{13}$C-NMR SPECTRUM OF COMPOUND B3

Current Data Parameters

| | |
|---|---|
| NAME | A4598 |
| EXPNO | 2 |
| PROCNO | 1 |

F2 - Acquisition Parameters

| | |
|---|---|
| Date_ | 20080903 |
| Time | 14.21 |
| INSTRUM | qnx400 |
| PROBHD | 5 mm Multinuc |
| PULPROG | zgpg30 |
| TD | 32768 |
| SOLVENT | Aceton |
| NS | 2463 |
| DS | 2 |
| SWH | 24154.590 Hz |
| FIDRES | 0.737140 Hz |
| AQ | 0.6783476 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 | 100.6253045 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 | 100.6152801 MHz |
| WDW | EM |
| SSB | 0 |
| LB | 2.50 Hz |
| GB | 0 |
| PC | 1.40 |

1D NMR plot parameters

| | |
|---|---|
| CX | 20.00 cm |
| F1P | 47.727 ppm |
| F1 | 4802.04 Hz |
| F2P | 6.377 ppm |
| F2 | 641.66 Hz |
| PPMCM | 2.06747 ppm/cm |
| HZCM | 208.01900 Hz/cm |

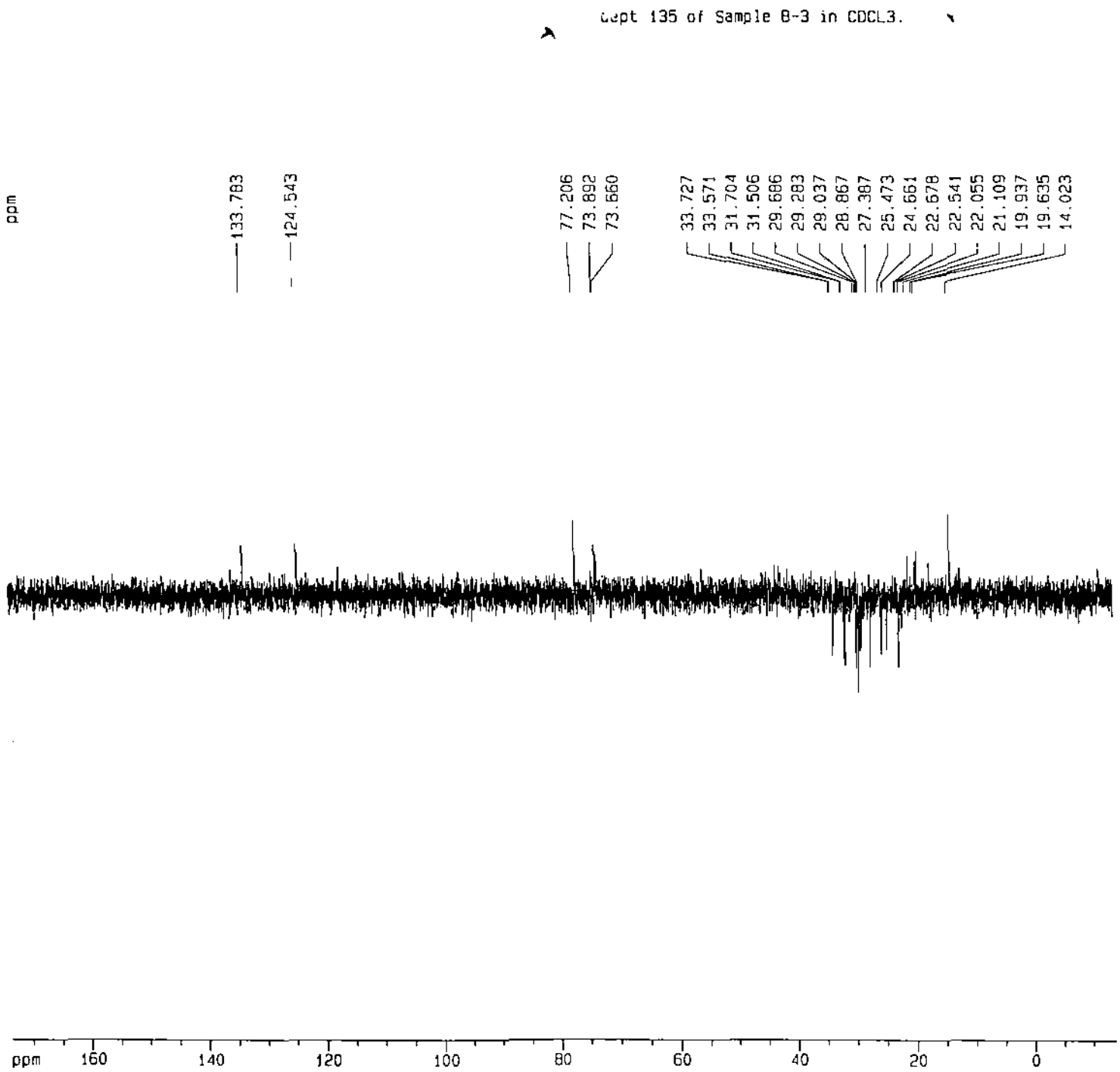



FIG 5.2.9 DEPT-135 SPECTRUM OF COMPOUND B3

Current Data Parameters

| | |
|---|---|
| NAME | A4598 |
| EXPNO | 3 |
| PROCNO | 1 |

F2 - Acquisition Parameters

| | |
|---|---|
| Date_ | 20080907 |
| Time | 10.58 |
| INSTRUM | dpx400 |
| PROBHD | 5 mm Multinuc |
| PULPROG | dept135 |
| TD | 32768 |
| SOLVENT | CDCl3 |
| NS | 1393 |
| DS | 8 |
| SWH | 24154.590 Hz |
| FIDRES | 0.737140 Hz |
| AQ | 0.6783476 sec |
| RG | 13004 |
| DW | 20.700 usec |
| DE | 6.00 usec |
| TE | 300.0 K |
| CNST2 | 145.0000000 |
| D1 | 4.00000000 sec |
| d2 | 0.00344828 sec |
| d12 | 0.00002000 sec |
| DELTA | 0.00000764 sec |

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

| | |
|---|---|
| NUC1 | 13C |
| P1 | 6.00 usec |
| p2 | 12.00 usec |
| PL1 | -6.00 dB |
| SFO1 | 100.6253045 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 | 100.6152828 MHz |
| WDW | EM |
| SSB | 0 |
| LB | 1.00 Hz |
| GB | 0 |
| PC | 1.40 |

1D NMR plot parameters

| | |
|---|---|
| CX | 20.00 cm |
| F1P | 173.717 ppm |
| F1 | 17478.56 Hz |
| F2P | -13.797 ppm |
| F2 | -1388.20 Hz |
| PPMCM | 9.37569 ppm/cm |
| HZCM | 943.33813 Hz/cm |

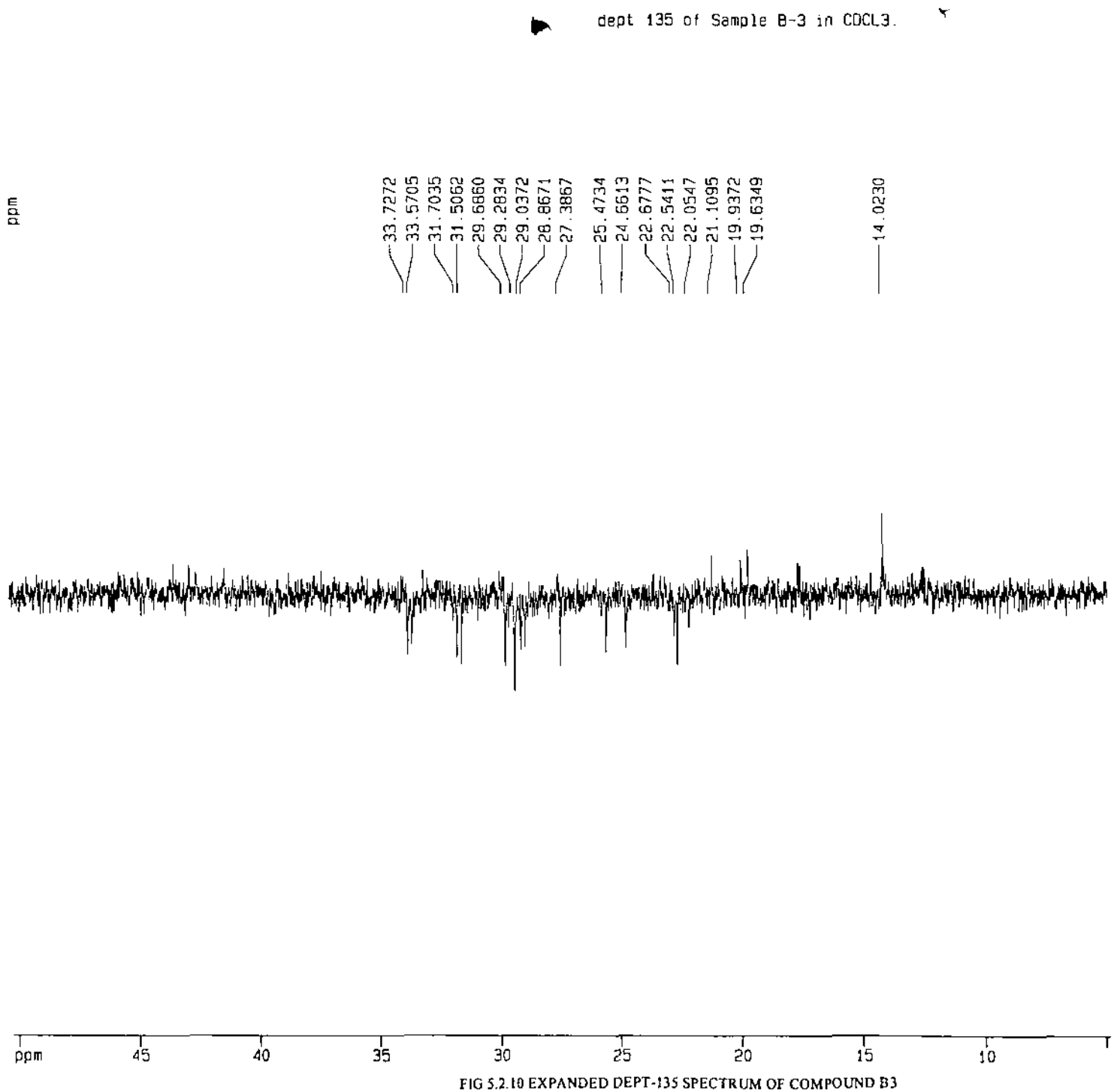



FIG 5.2.10 EXPANDED DEPT-135 SPECTRUM OF COMPOUND B3

Current Data Parameters

| | |
|---|---|
| NAME | A4598 |
| EXPNO | 3 |
| PROCNO | 1 |

F2 - Acquisition Parameters

| | |
|---|---|
| Date_ | 20080907 |
| Time | 10.58 |
| INSTRUM | dpx400 |
| PROBHD | 5 mm Multinuc |
| PULPROG | dept135 |
| TD | 32768 |
| SOLVENT | CDCl3 |
| NS | 1393 |
| DS | 8 |
| SWH | 24154.590 Hz |
| FIDRES | 0.737140 Hz |
| AQ | 0.6783476 sec |
| RG | 13004 |
| DW | 20.700 usec |
| DE | 6.00 usec |
| TE | 300.0 K |
| CNST2 | 145.0000000 |
| D1 | 4.00000000 sec |
| d2 | 0.00344828 sec |
| d12 | 0.00002000 sec |
| DELTA | 0.00000764 sec |

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

| | |
|---|---|
| NUC1 | 13C |
| P1 | 6.00 usec |
| p2 | 12.00 usec |
| PL1 | -6.00 dB |
| SFO1 | 100.6253045 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 | 100.6152828 MHz |
| WDW | EM |
| SSB | 0 |
| LB | 1.00 Hz |
| GB | 0 |
| PC | 1.40 |

1D NMR plot parameters

| | |
|---|---|
| CX | 20.00 cm |
| F1P | 50.238 ppm |
| F1 | 5054.73 Hz |
| F2P | 4.827 ppm |
| F2 | 485.64 Hz |
| PPMCM | 2.27058 ppm/cm |
| HZCM | 228.45468 Hz/cm |

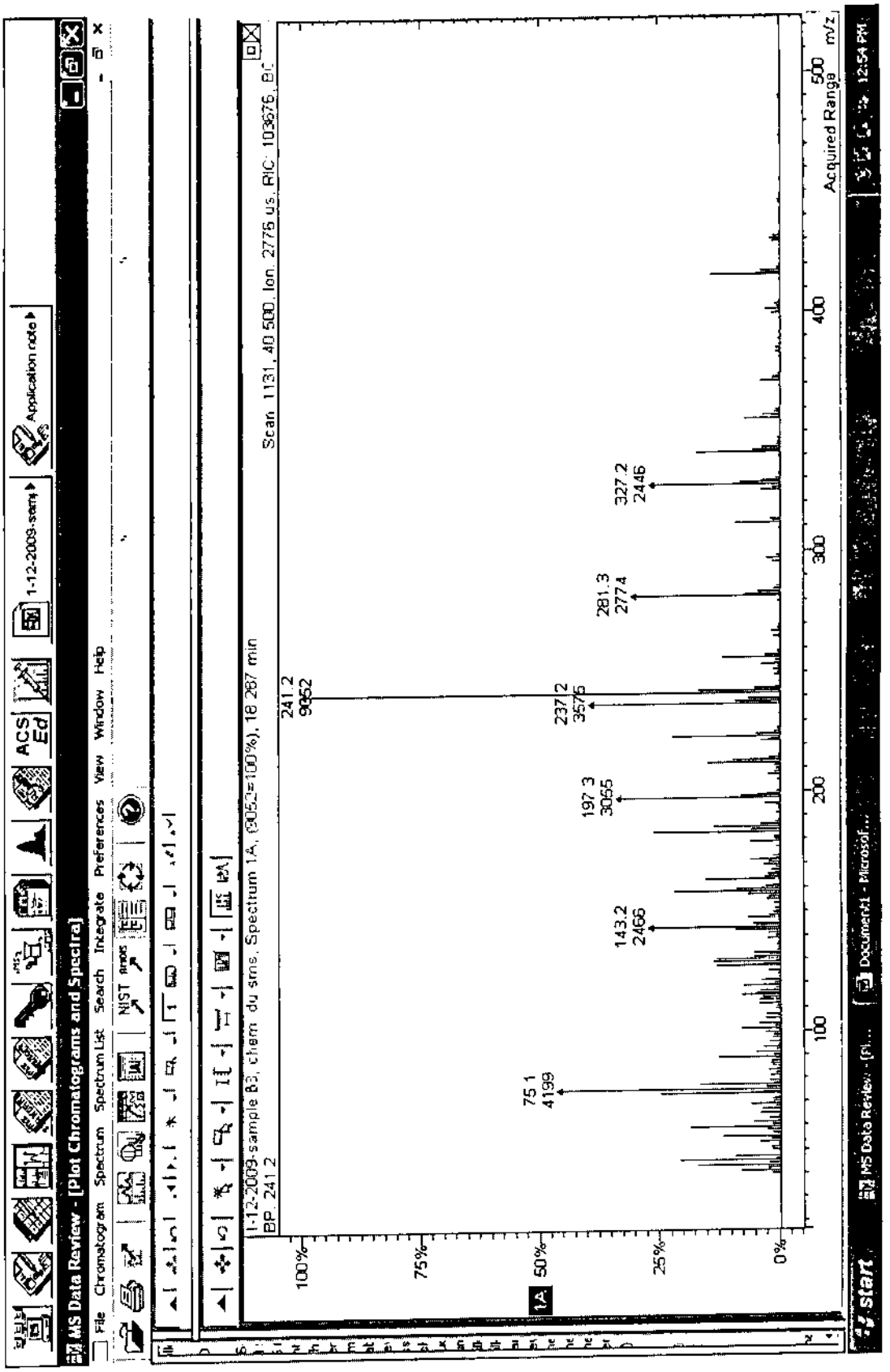



FIG 5.2.11 GC-MS SPECTRUM OF COMPOUND B3

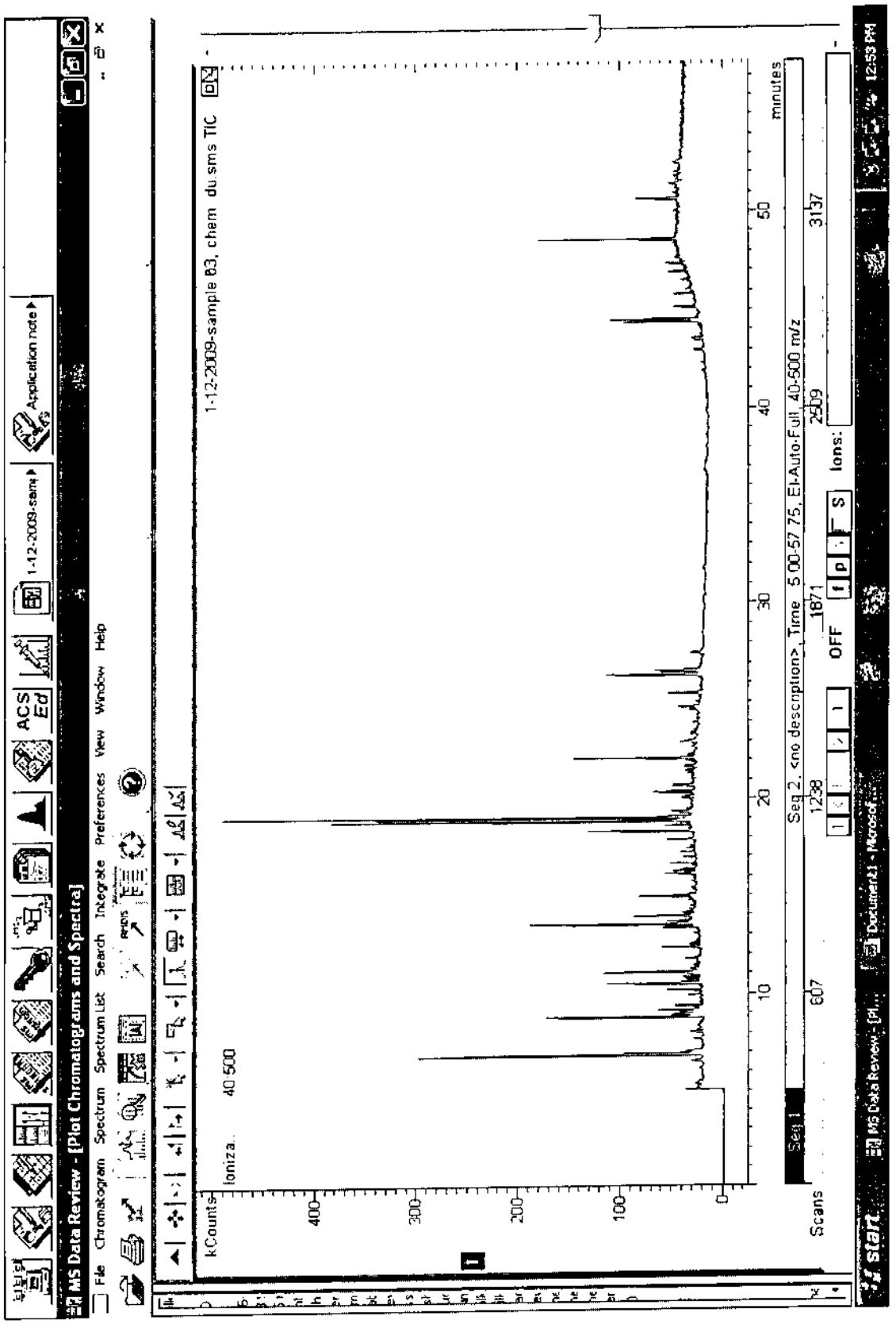



FIG 5.2.12 GC-MS SPECTRUM OF COMPOUND B3

### 5.2.4 MASS SPECTROSCOPIC ANALALYSIS OF COMPOUND B3

The compound contained m/z =415 assigned to the molecular ion of the compound B3. However other weak peaks were also in the chromatogram, which we could not explained.

Combining *IR*, $^{1}H$-*NMR*, $^{13}C$-*NMR*, *DEPT NMR* and *GC-MS* spectroscopic data, the compound B3 was identified as trans-4-Acetoxy-5-hydroxy-5-pnntadecanoyl-2-cyclopentene-1-one having the structure as given below:

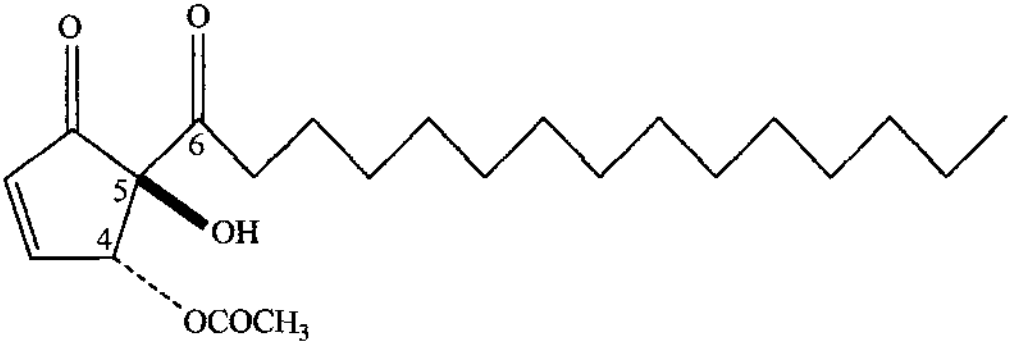



Fig 5.2.13 trans-4-Acetoxy-5-hydroxy-5-pentadecanoyl-2-cyclopenten-1-one

## 5.3 CHARACTERISATION OF COMPOUND B6

The compound B6 (72.5 mg) was isolated as reddish colour substance from the dichloromethane extract of the plant *Pleurotus ostreatus* upon vacuum liquid chromatography from the fraction F6. It was soluble in dichloromethane . Its TLC study indicated the presence of a single spot ($R_f$= 0.45) in over silica gel GF254, dichloromethane: methanol =95:05 as the mobile phase. The spot was visualized as a black colour upon spraying with vanillin-sulfuric acid followed by heating in an oven at 110°C.

### 5.3.1 IR SPECTROSCOPIC ANALYSIS OF COMPOUND B6

The IR spectrum (Fig-5.3.1 ) of the compound B6 showed an absorption band at 3400 $cm^{-1}$ indicating the presence of -OH group. The band at 2929 $cm^{-1}$ was for C-H stretching and 1359 $cm^{-1}$ for $-CH_3$ group. The peak at 1637 $cm^{-1}$ was suggestive the presence of >C=C< group. The band at 1425 $cm^{-1}$ indicated $-CH_2$-group. The absorption band at 1710 $cm^{-1}$ was suggestive of C=O stretching. The other peak at 720 $cm^{-1}$ indicated the presence of long chain band.

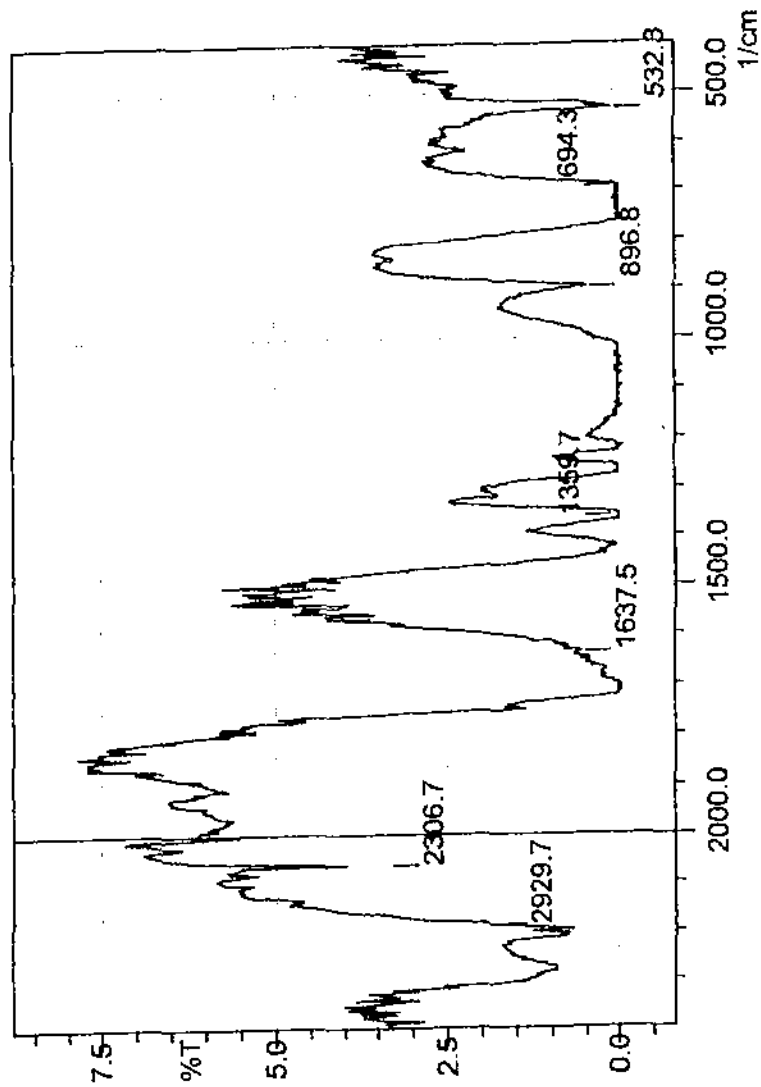



FIG 5.3.1 IR SPECTRUM OF COMPOUND B6

## 5.3.2 $^1$H-NMR SPECTROSCOPIC ANALYSIS OF COMPOUND B6

The $^1$H-NMR (400MHz in $CDCl_3$) spectrum (Fig-5.3.2-5.3.4) of the isolated compound B6 showed a downfield at chemilal shift, δ 5.581 and 7.249 ppm suggestive of the presence of olefinic proton (2-H and 3-H).[116]

The $^1$H-NMR spectrum displayed at δ 5.179 ppm for the proton at C-6. The spectrum showed a band at δ 3.310 ppm suggestive of an oxymethine proton 5-H.

The proton of acetoxy group at C-4 and C-6 showed signal at δ 2.155 ppm and δ 2.032 ppm.Methelene proton at C-7 showed peak at δ 1.620 ppm and methyl proton at C-20 showed peak at δ 0.874 ppm. The chemilal shift, δ 1.20-1.469 ppm showed signals for the methelene proton at C-8 and C-19.

**Table-5.5: $^1$H-NMR spectral data of compound B6**

| No.of protons | Chemical shift value(δ) | |
|---|---|---|
| | Experiment value(δ) | Reported value[116] (δ) |
| H-3 | 7.249 | 7.462(dd, J=6.2/2.1 Hz) |
| H-2 | 5.581 | 6.441( dd, J=6.2/1.8) |
| H-4 | 5.581 | 5.721(dd, J=2.1/1.8) |
| H-6 | 5.171 | 5.017(m) |
| 5-OH | 3.310 | 3.247(s) |
| 4-OAc | 2.155 | 2.136(s) |
| 6-OAc | 2.032 | 1.996(s) |
| H-7 | 1.620 | 1.7(m) |
| H-8-H-19 | 1.20-1.469 | 1.20-1.33(m) |
| H-20 | 0.874 | 0.888 (t) |

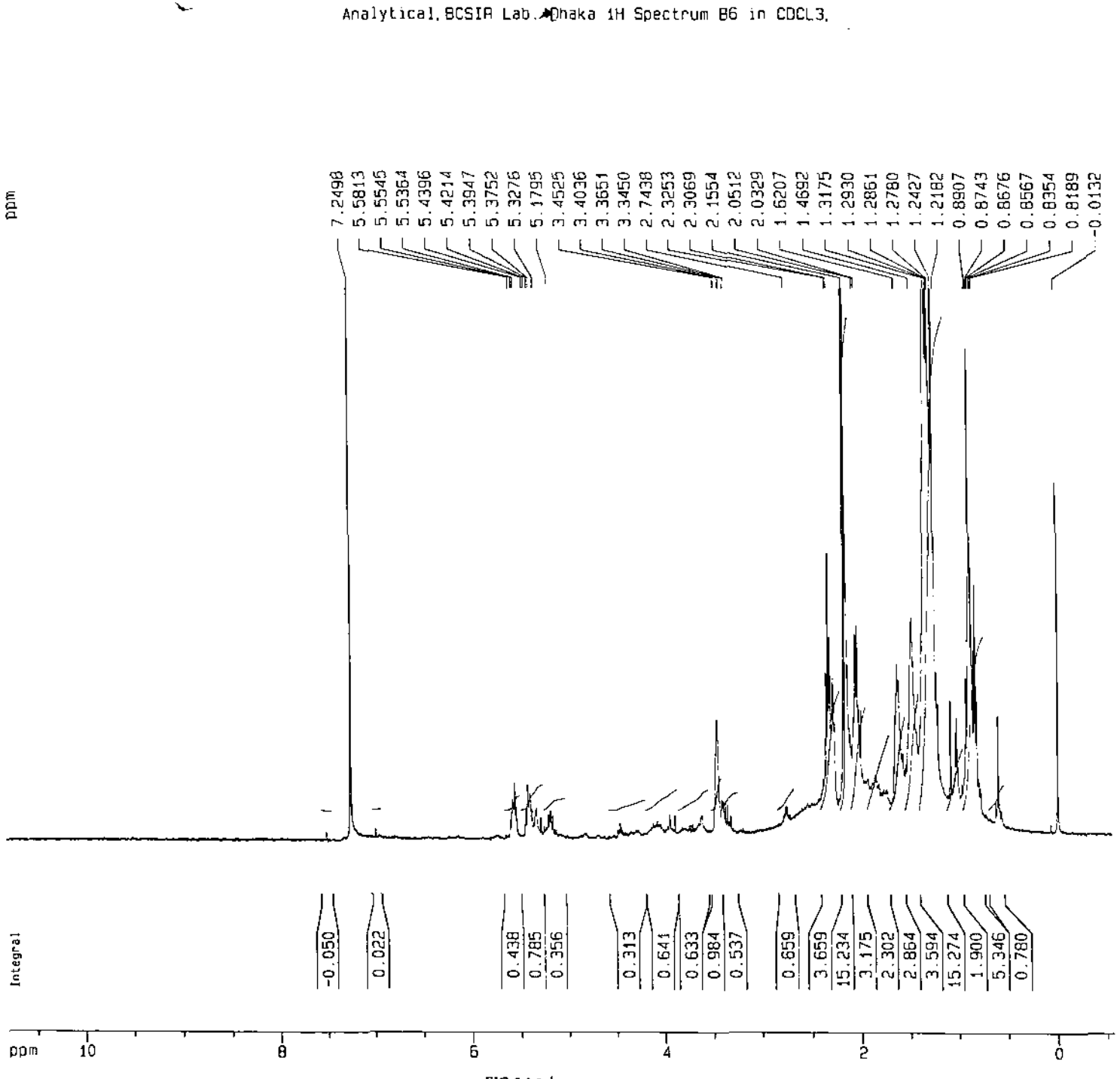



FIG 5.3.2 $^{1}$H-NMR SPECTRUM OF COMPOUND B6

Current Data Parameters

| | |
|---|---|
| NAME | A4600 |
| EXPNO | 1 |
| PROCNO | 1 |

F2 - Acquisition Parameters

| | |
|---|---|
| Date_ | 20080903 |
| Time | 12.36 |
| INSTRUM | dpx400 |
| PROBHD | 5 mm Multinuc |
| PULPROG | zg30 |
| TD | 32768 |
| SOLVENT | Aceton |
| NS | 128 |
| DS | 2 |
| SWH | 6410.256 Hz |
| FIDRES | 0.195625 Hz |
| AQ | 2.5559540 sec |
| RG | 287.4 |
| DW | 78.000 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 | 400.1428010 MHz |

F2 - Processing parameters

| | |
|---|---|
| SI | 32768 |
| SF | 400.1400126 MHz |
| WDW | EM |
| SSB | 0 |
| LB | 0.30 Hz |
| GB | 0 |
| PC | 1.40 |

1D NMR plot parameters

| | |
|---|---|
| CX | 20.00 cm |
| F1P | 10.807 ppm |
| F1 | 4324.48 Hz |
| F2P | -0.565 ppm |
| F2 | -226.06 Hz |
| PPMCM | 0.56862 ppm/cm |
| HZCM | 227.52664 Hz/cm |

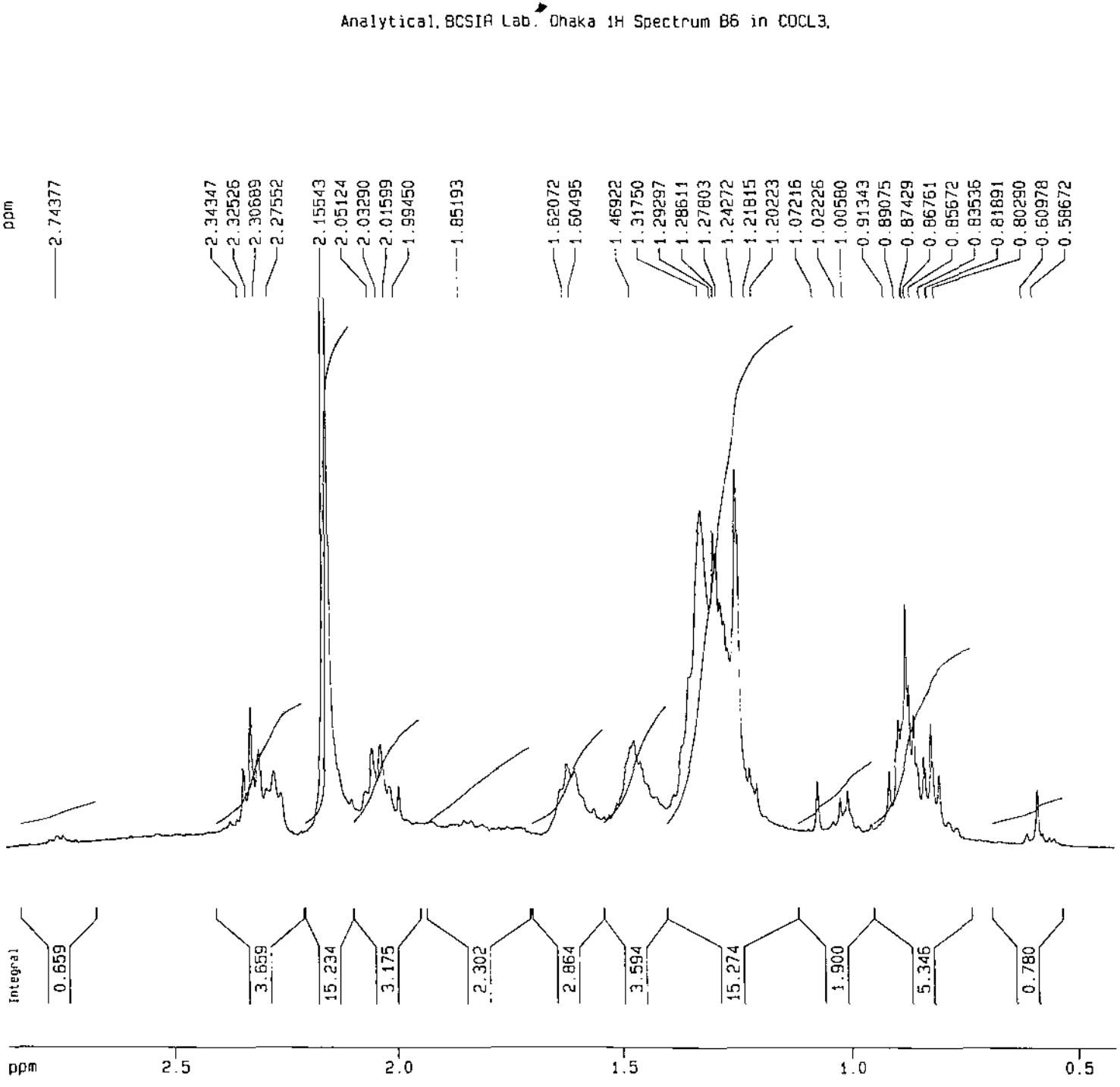



FIG 5 3.3 EXPANDED $^{1}$H-NMR SPECTRUM OF COMPOUND B6

Current Data Parameters

| | |
|---|---|
| NAME | A4600 |
| EXPNO | 1 |
| PROCNO | 1 |

F2 - Acquisition Parameters

| | |
|---|---|
| Date_ | 20080903 |
| Time | 12.36 |
| INSTRUM | dpx400 |
| PROBHD | 5 mm Multinuc |
| PULPROG | zg30 |
| TD | 32768 |
| SOLVENT | Aceton |
| NS | 128 |
| DS | 2 |
| SWH | 6410.256 Hz |
| FIDRES | 0.195625 Hz |
| AQ | 2.5559540 sec |
| RG | 287.4 |
| DW | 78.000 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 | 400.1428010 MHz |

F2 - Processing parameters

| | |
|---|---|
| SI | 32768 |
| SF | 400.1400126 MHz |
| WDW | EM |
| SSB | 0 |
| LB | 0.30 Hz |
| GB | 0 |
| PC | 1.40 |

1D NMR plot parameters

| | |
|---|---|
| CX | 20.00 cm |
| F1P | 2.869 ppm |
| F1 | 1148.14 Hz |
| F2P | 0.419 ppm |
| F2 | 167.47 Hz |
| PPMCM | 0.12254 ppm/cm |
| HZCM | 49.03355 Hz/cm |

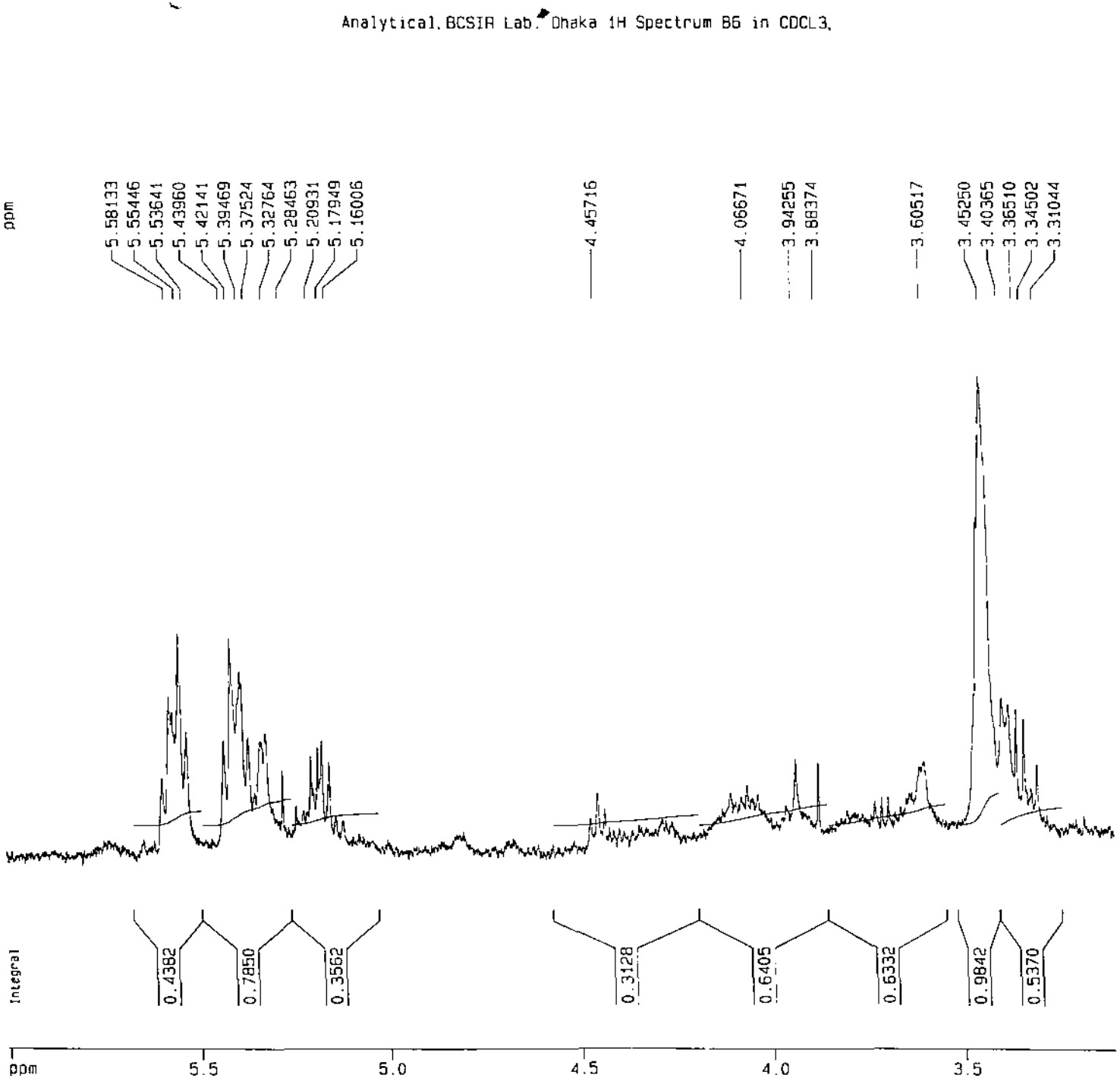



FIG 5.3.4 EXPANDED $^{1}$H-NMR SPECTRUM OF COMPOUND B6

Current Data Parameters

| | |
|---|---|
| NAME | A4600 |
| EXPNO | 1 |
| PROCNO | 1 |

F2 - Acquisition Parameters

| | |
|---|---|
| Date_ | 20080903 |
| Time | 12.36 |
| INSTRUM | dpx400 |
| PROBHD | 5 mm Multinuc |
| PULPROG | zg30 |
| TD | 32768 |
| SOLVENT | Aceton |
| NS | 128 |
| DS | 2 |
| SWH | 6410.256 Hz |
| FIDRES | 0.195625 Hz |
| AQ | 2.5559540 sec |
| RG | 287.4 |
| DW | 78.000 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 | 400.1428010 MHz |

F2 - Processing parameters

| | |
|---|---|
| SI | 32768 |
| SF | 400.1400126 MHz |
| WDW | EM |
| SSB | 0 |
| LB | 0.30 Hz |
| GB | 0 |
| PC | 1.40 |

1D NMR plot parameters

| | |
|---|---|
| CX | 20.00 cm |
| F1P | 6.009 ppm |
| F1 | 2404.53 Hz |
| F2P | 3.110 ppm |
| F2 | 1244.27 Hz |
| PPMCM | 0.14498 ppm/cm |
| HZCM | 58.01279 Hz/cm |

### 5.3.3 $^{13}$C-NMR SPECTROSCOPIC ANALYSIS OF COMPOUND B6

The $^{13}$C-NMR spectrum (Fig-5.3.5-5.3.7) of the isolated compound B6 revealed the presence of attributed to 24 carbon atom. The signal at chemical shift,δ 14.039 ppm (C-20) was assigned to a methyl carbon. The signals at chemical shift,δ 22.562 ppm (C-19), δ 24.682 ppm (C-18), δ 27.404 ppm (C-17), δ 28.906 ppm (C-16), δ 29.078 ppm (C-15), δ 29.306 ppm (C-14), δ 29.506 ppm (C-13), δ 29.722 ppm (C-12), δ 30.911 ppm (C-11), δ 31.526 ppm (C-10), δ 31.732 ppm (C-9), δ 33.601 ppm (C-8) were assigned for methylene carbons respectively. The signal at δ 124.568 ppm (C-2) and δ 133.812 ppm (C-3) were assigned for the >C=C carbons. The signal at δ 22.562 ppm was assigned for acetoxy methyl carbon attached to C-4. δ 76.712 ppm and δ 77.029 ppm were assigned for CH attached to C-6 and C-4 respectively.. The signal at δ 206.958 ppm was due to the presence of >C=O attached to C-1.

**Table-5.6: $^{13}$C-NMR spectral data of the isolated compound B6**

| No. of carbon | Types of carbon (assigned) | Chemical shift value (Observed)[116] |
|---|---|---|
| C-20 | Methyl carbon | 14.039 |
| C-19 | Methylene carbon | 22.562 |
| C-18 | Methylene carbon | 24.682 |
| C-17 | Methylene carbon | 27.404 |
| C-16 | Methylene carbon | 28.906 |
| C-15 | Methylene carbon | 29.078 |
| C-14 | Methylene carbon | 29.306 |
| C-13 | Methylene carbon | 29.506 |
| C-12 | Methylene carbon | 29.722 |
| C-11 | Methylene carbon | 30.911 |
| C-10 | Methylene carbon | 31.526 |
| C-9 | Methylene carbon | 31.732 |
| C-8 | Methyne carbon | 33.601 |
| Acetoxy methyl carbon at C-4 | Acetoxy methyl carbon | 22.562 |
| C-7 | $CH_2$ | 28.906 |
| C-6 | CH | 76.712 |
| C-4 | CH | 77.029 |
| C-5 | Quartenary carbon | 77.347 |
| C-2 | Olefinic | 124.568 |
| C-3 | Olefinic | 133.812 |
| C-1 | Ketone carbon | 206.958 |

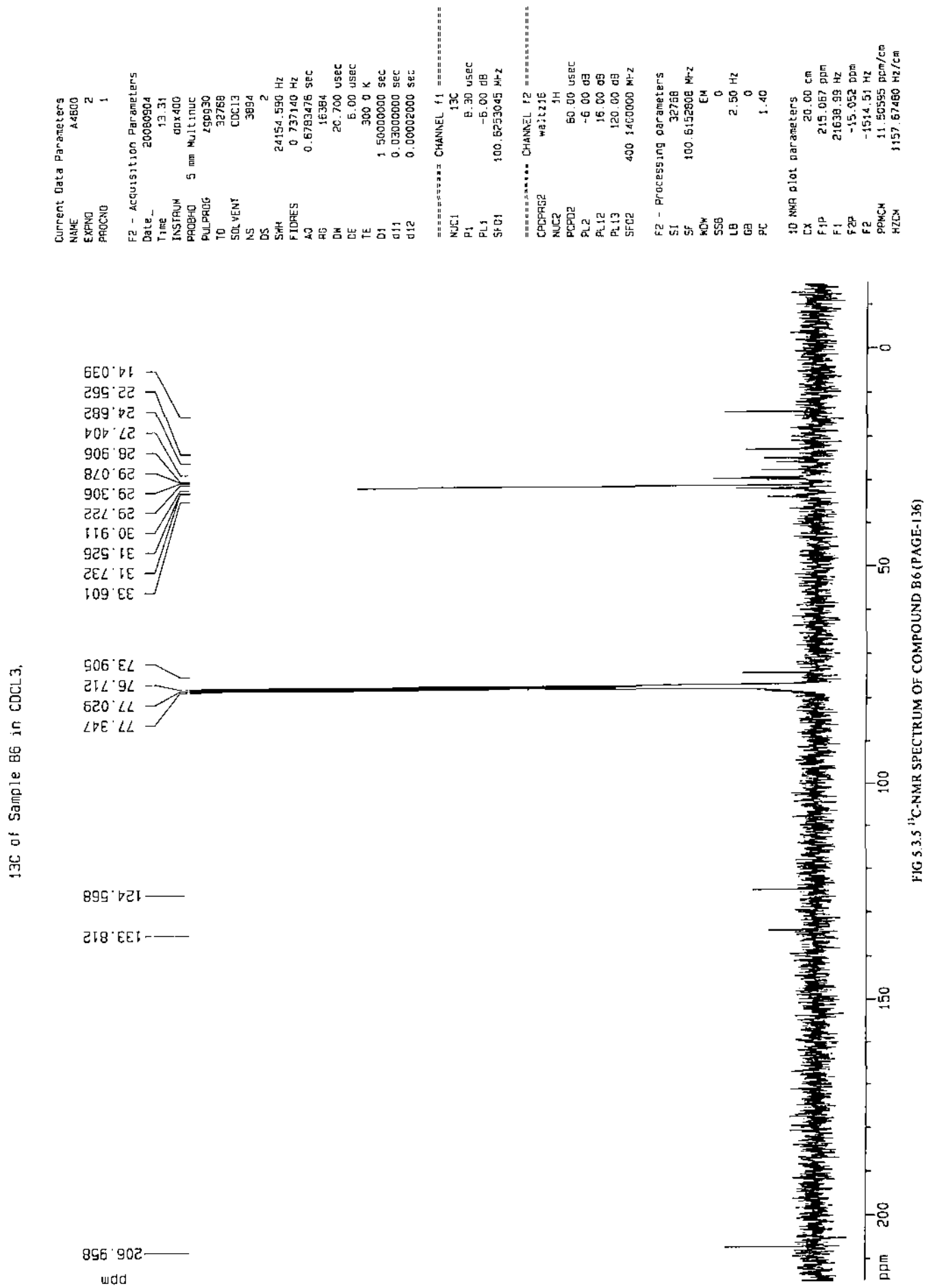
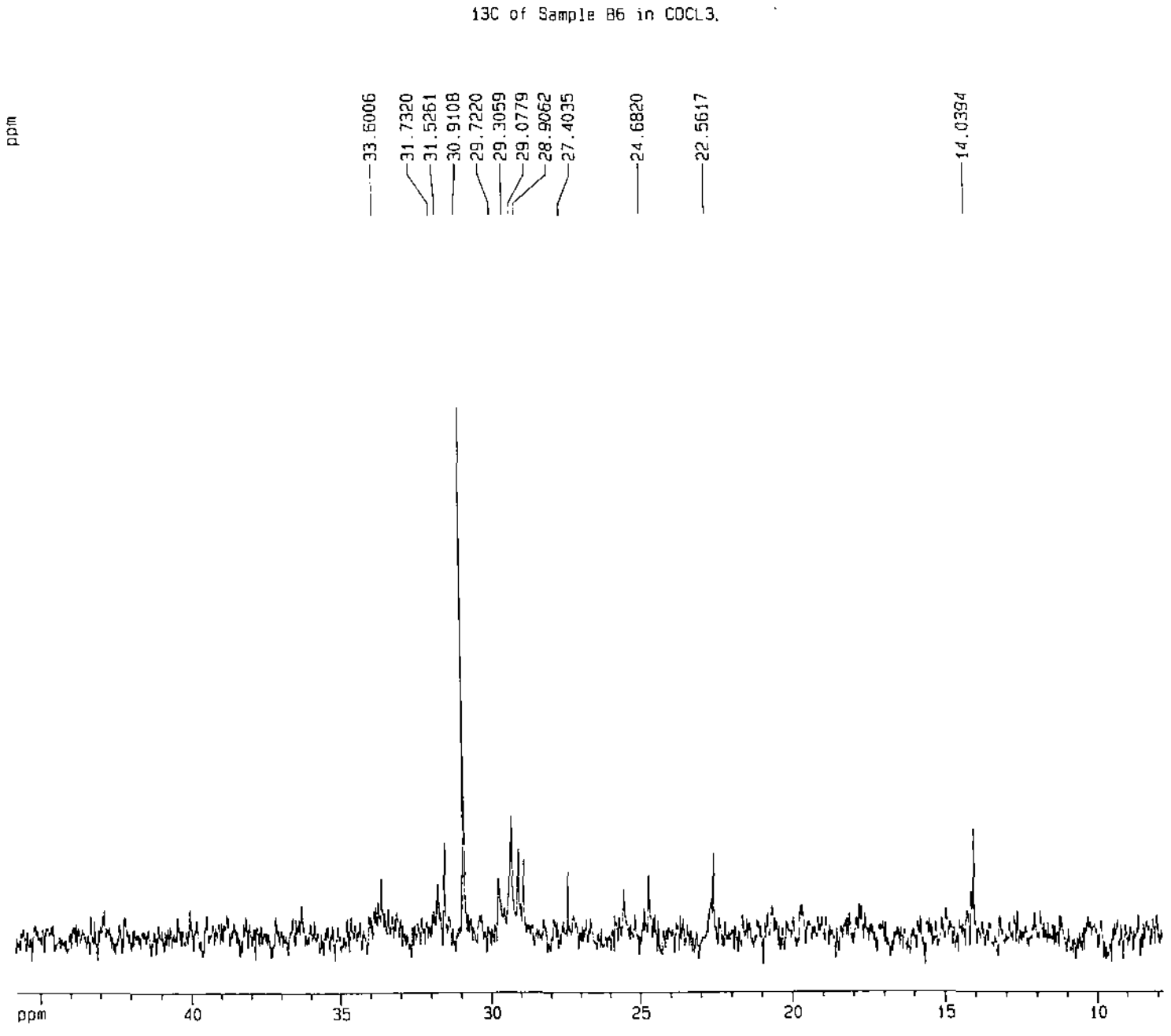



FIG 5.3.5 $^{13}$C-NMR SPECTRUM OF COMPOUND B6 (PAGE-136)




Current Data Parameters
NAME A4600
EXPNO 2
PROCNO 1

F2 - Acquisition Parameters
Date_ 20080904
Time 13.31
INSTRUM dpx400
PROBHD 5 mm Multinuc
PULPROG zgpg30
TD 32768
SOLVENT CDCl3
NS 3894
DS 2
SWH 24154.590 Hz
FIDRES 0.737140 Hz
AQ 0.6783476 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 100.6253045 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 100.6152808 MHz
WDW EM
SSB 0
LB 2.50 Hz
GB 0
PC 1.40

1D NMR plot parameters
CX 20.00 cm
F1P 45.805 ppm
F1 4608.72 Hz
F2P 7.827 ppm
F2 787.50 Hz
PPMCM 1.89893 ppm/cm
HZCM 191.06134 Hz/cm

FIG 5.3.6 EXPANDED $^{13}$C-NMR SPECTRUM OF COMPOUND B6

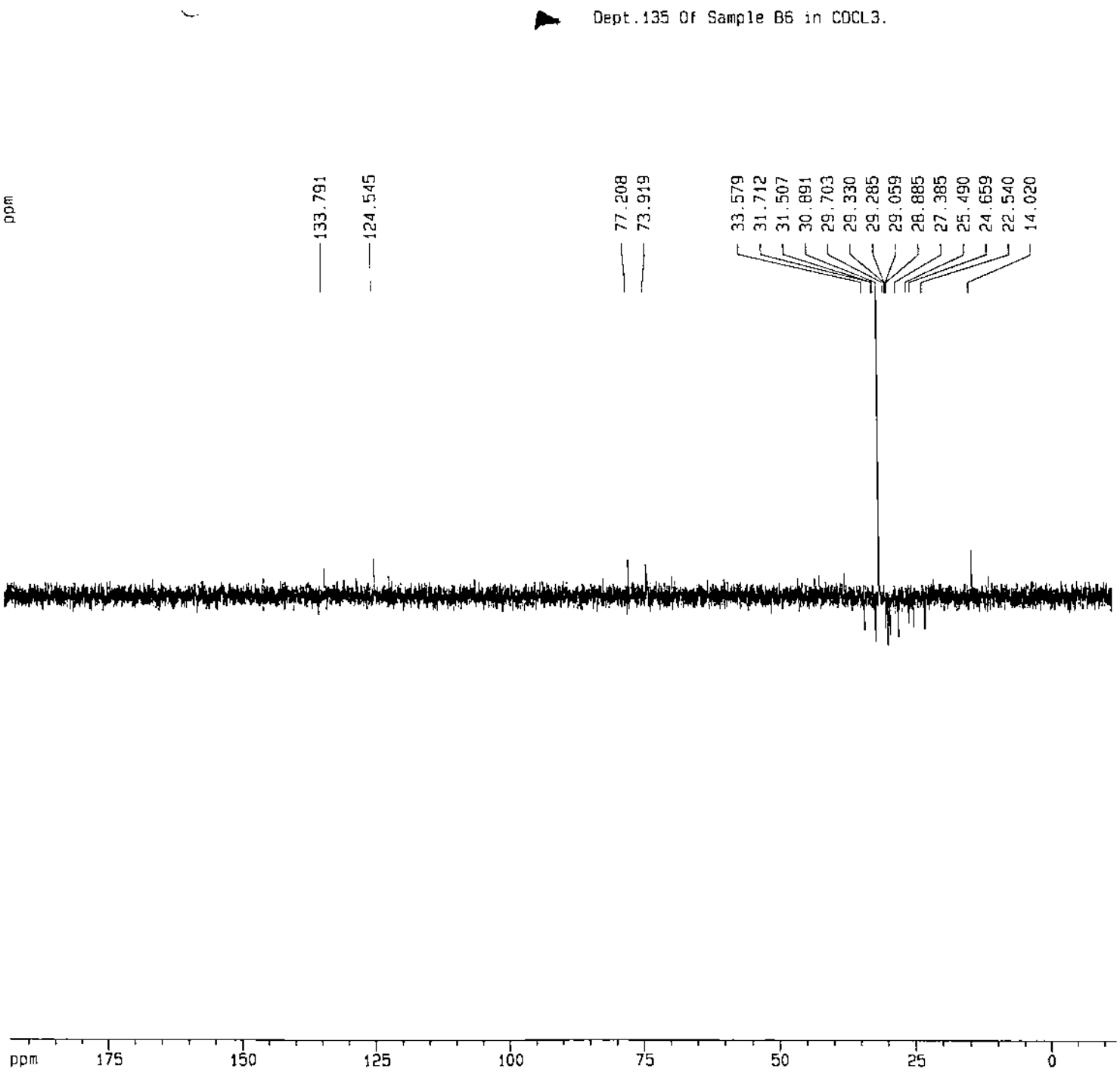



FIG 5.3.7 DEPT-135 SPECTRUM OF COMPOUND B6

Current Data Parameters

| | |
|---|---|
| NAME | A4600 |
| EXPNO | 3 |
| PROCNO | 1 |

F2 - Acquisition Parameters

| | |
|---|---|
| Date_ | 20080904 |
| Time | 15.12 |
| INSTRUM | dpx400 |
| PROBHD | 5 mm Multinuc |
| PULPROG | dept135 |
| TD | 32768 |
| SOLVENT | CDCl3 |
| NS | 671 |
| DS | 8 |
| SWH | 24154.590 Hz |
| FIDRES | 0.737140 Hz |
| AQ | 0.6783476 sec |
| RG | 13004 |
| DW | 20.700 usec |
| DE | 6.00 usec |
| TE | 300.0 K |
| CNST2 | 145.0000000 |
| D1 | 4.00000000 sec |
| d2 | 0.00344828 sec |
| d12 | 0.00002000 sec |
| DELTA | 0.00000764 sec |

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

| | |
|---|---|
| NUC1 | 13C |
| P1 | 6.00 usec |
| p2 | 12.00 usec |
| PL1 | -6.00 dB |
| SF01 | 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 |
| SF02 | 400.1420067 MHz |

F2 - Processing parameters

| | |
|---|---|
| SI | 32768 |
| SF | 100.6152828 MHz |
| WDW | EM |
| SSB | 0 |
| LB | 1.00 Hz |
| GB | 0 |
| PC | 1.40 |

1D NMR plot parameters

| | |
|---|---|
| CX | 20.00 cm |
| F1P | 193.616 ppm |
| F1 | 19480.74 Hz |
| F2P | -12.266 ppm |
| F2 | -1234.19 Hz |
| PPMCM | 10.29413 ppm/cm |
| HZCM | 1035.74683 Hz/cm |

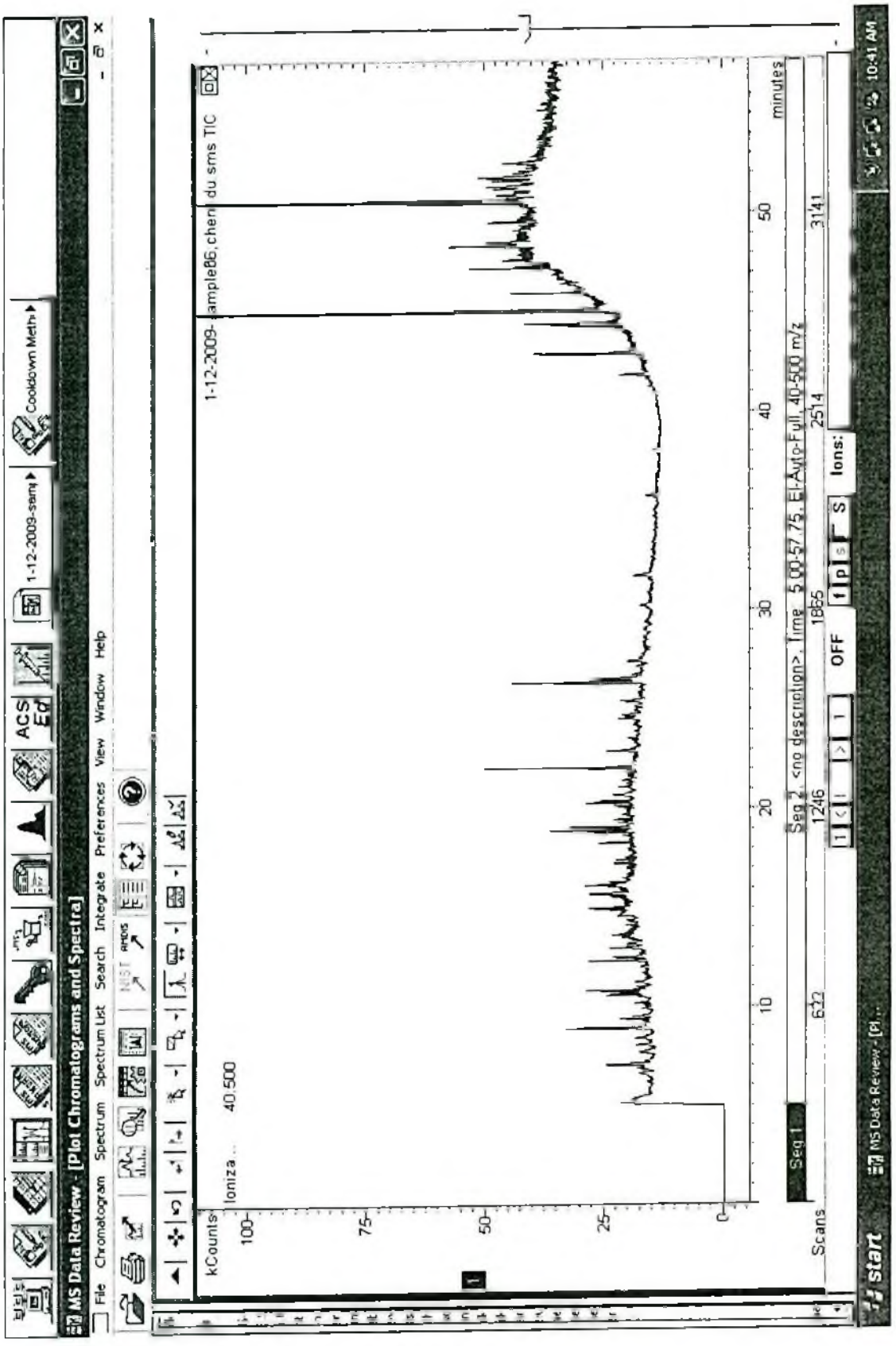



FIG 5.3.8 GC-MS SPECTRUM OF COMPOUND B6

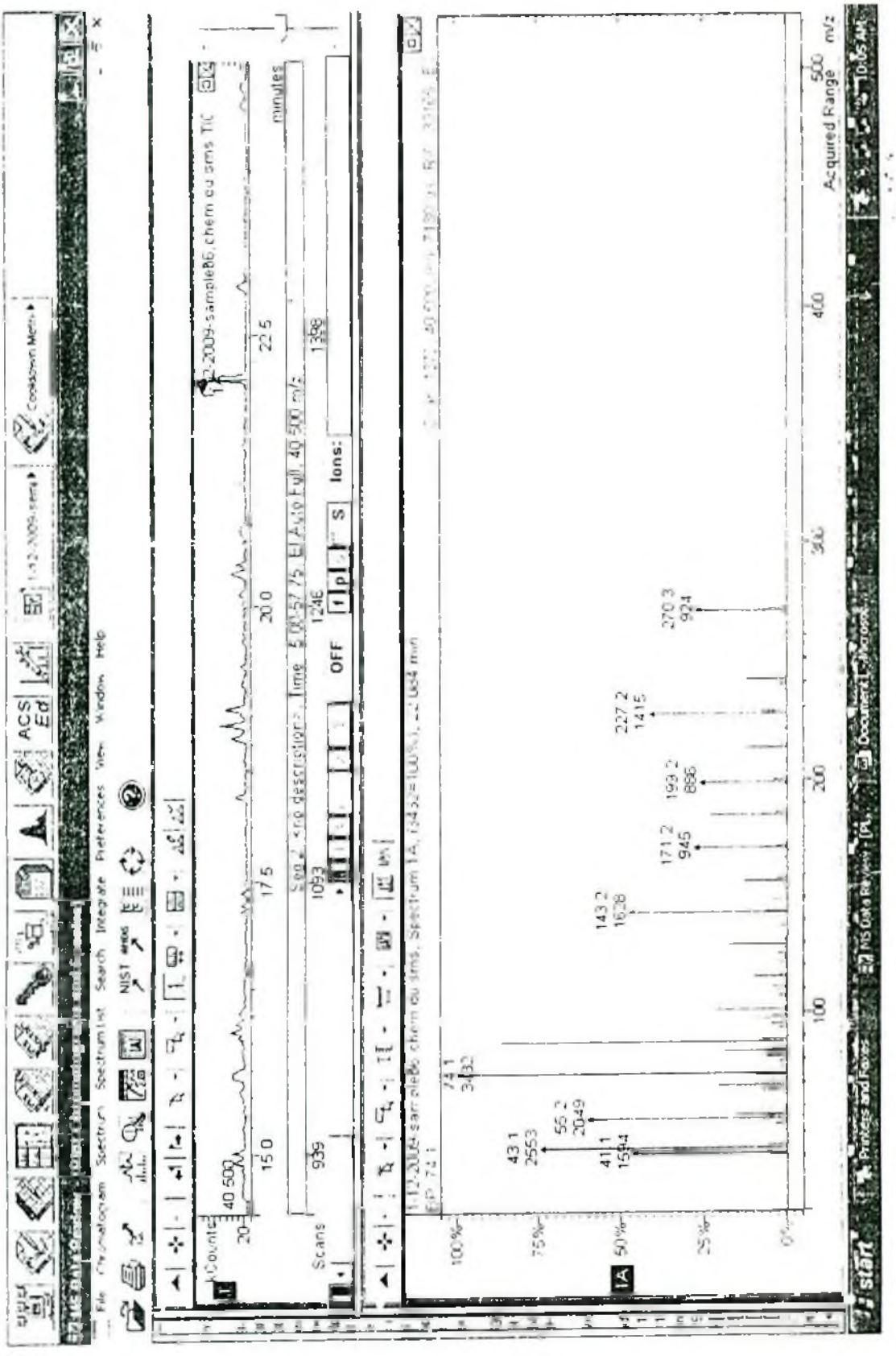



**FIG 5.3.9 GC-MS SPECTRUM OF COMPOUND B6.**

### 5.3.4 MASS SPECTROSCOPIC ANALALYSIS OF COMPOUND B6

The compound contained m/z =270 assigned for of the compound B3. However other weak peaks were also in the chromatogram, which we could not explained.

Combining IR, $^{1}$H-NMR, $^{13}$C-NMR, DEPT NMR and GC-MS spectroscopic data, the compound B6 was identified as 4,5-trans-4-Acetoxy-5-hydroxy-5-(1-acetoxypentadecyl)-2-cyclopentene-1-one having the structure as given below:

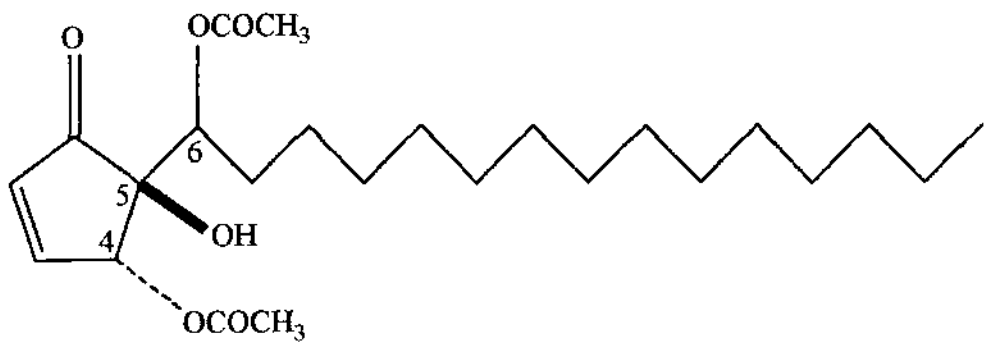



Fig-5.3.10 4,5-trans-4-Acetoxy-5-hydroxy-5-(1-acetoxypentadecyl)-2- cyclopentene- 1-one

# SUMMARY

## SUMMARY

Mushrooms are plant like organism that lack chlorophyll. Mushrooms belonging to the *genus*, more known as oyster mushrooms, alluding to its appearance, have brought great interest due to the varsality of their crop. A wide range of substrates are employed including agroindustrial waste for their production. Mushrooms considered as a nutritious and delicious food since earliest time and recognized as a distinct source of vegetable protein. FAO has recommended edible mushroom as a source of protein nutrition of developing countries where malnutrition is a serious problem (Bahl, 1994). Mushrooms contain 19-35% proteins on dry weight basis as compared to 7.3% in rice, 13.2% in wheat, and 25.2% in milk (Chang and Miles, 1988). Mushroom contains all the nine essential amino acids to a considerable amount. Carbohydrate content is reported in the range of 46.6 to 81.1 percent in *Pleurotus* species (Bano and Rajarathnam, 1982).

The fruitbody of mushroom "*Pleurotus ostreatus*" have been collected from the National Mushroom Development and Extend Center, Savar of Dhaka district and these were identified from the department of Botany, Dhaka University. It was dried under mild sunlight and then at $40^0$ C in an oven. Afterwards the mushrooms were powdered in a grinding machine (~ 200 mesh). The powder was used throughout the investigation.

The percentages of ash and moisture in this species of *pleurotus ostreatus* were determined by standard method and they were found to be 14.89 % and 8.70 % respectively in dry powder basis. The acid value, saponification value and iodine values are 4.58, 260 and 158 respectively.

Fatty acids (free fatty acids) of the *pleurotus ostreatus* were analyzed. The compositional analysis of n-Hexane extract of *pleurotus ostreatus* by GC revealed that the free fatty

acid portion of this species was rich in unsaturated fatty acids (such as capric acid, Stearic acid, Oleic acid, Palmitic acid, Lauric acid ).

Phytochemical screening of this plant was carried out from its aqueous extract and taking the powdered specimens using standard procedures. This screening indicated the presence of Alkaloid, Steroid and Terpenoid.

Mushroom powdered sample was extracted with n-Hexane and followed by dichloromethane exhaustively. The extracts were concentrated separated to a dry mass. The dry mass (0.67 g) of the dichloromethane extract was subjected to VLC for separation of different compounds.

The fractions obtained from dichloromethane extract by VLC using different ratio of elutions were marked as F1, F2, F3, F4, F5, F6, F7 and F8.

Fraction F4 was left undisturbed at room temperature for several days. A white needle shaped crystal was obtained. The crystals were washed with solvents. After washing, the solid was dissolved in dichloromethane and the solution was kept in a vial for recrystallization at room temperature and crystals were obtained. Its TLC study also showed a single spot in solvent system, dichloromethane 100%.The spot was pink colour by spraying with vanillin sulfuric acid. Its TLC study also showed a single spot in different solvent systems. Its melting point is $122^0$-$124^0$C.

Attempt was taken to characterize this Compound F4 (78.9 mg) by melting point, IR , $^1$H-NMR , $^{13}$C-NMR, DEPT and mass spectral data and it was tentatively suggested to be ergosta-5,7,22-trien-β-ol (ergosterol) isostearate.

The fraction F6 was concentrated to a small volume and was left undisturbed at room temperature. After several days a solid substance was obtained. The TLC study in solvent (DCM:MeOH=90:10) showed the presence of two prominent bright spots. Solid was placed on the top of the bed of silica gel packed column. The column was first eluted with mixture of dicholoromethane and increasing amount of methanol. The eluents were collected in an amount of about 5mL in a series of test tubes. As a result of elution in sub column gave B3 and B6 fraction. They were soluble in dichloromethane. It showed single spot on tlc and uses characterized as single compounds.

Combining IR, $^{1}$H-NMR, $^{13}$C-NMR, DEPT NMR and GC-MS spectroscopic data, the compound B3 (75.2 mg) was identified as trans-4-Acetoxy-5-hydroxy-5-pnntadecanoyl-2-cyclopentene-1-one.

Combining IR, $^{1}$H-NMR, $^{13}$C-NMR, DEPT NMR and GC-MS spectroscopic data, the compound B6 (72.5 mg) was identified as 4,5-trans-4-Acetoxy-5-hydroxy-5-(1-acetoxypentadecyl)-2-cyclopentene-1-one.

**REFERENCES**

1.Marmion, V J et. al. The Death of Claudius, The Royal Society of Medicine, © 2002

2.Rubel, William. Amanita Muscaria

3.Arora, David. Mushrooms Demystified, 2nd ed. Ten Speed Press, 1986

4.http://www.fao.org/docrep/007/y5489e/y5489e12.htm#P3401_301941Edible mushrooms by country, sept.07,2008

5.http://www.fao.org/docrep/007/y5489e/y5489e00.htm#TopOfPage Boa, E (2004) Wild Edible Fungi: a global overview of their uses and importance. FAO, Rome. sept.07,2008

6.Chang, S., and Miles, P.G., Mushrooms, Cultivation, Nutritional Value, Medicinal Effect, and Environmental Impact, CRC Press, 2004.

7.DURAND, R. Influence des radiations luminuses sur les processus de reproduction des *champignons: hypotheses sur lidentite des photorecepteurs: revue bibliographique.* Mycologia, v.60, p.3-16, 1976.

8.EGER, G.; GOTTWALD, H. D.; VON NETZER, U. The action of light and other factors on sporophore initiation in Pleurotus ostreatus. Mushroom Science, v.9, p.575-583, 1974.

9.CALLAC, P.; MOQUER, F.; IMBERNON, M.; GUEDES-LAFARGUE, M.R.; MAMOUN, M.; OLIVIER, J.M. Evidence for PPC1, a determinant of the pilei-pellis color of Agaricus bisporus fruitbodies. Fungal Genetics and Biology, v.23, p.181-188, 1998.

10.CHANG, S.T.; KWAN, H.S.; KANG, Y.N. Colletion, characterization, and utilization of germ plasm of Lentinula edodes. Canadian Journal of Botany, n.73, p.955-961, 1995, Supplement 1.

11.CELSO, P.G. Interações entre Agaricus bisporus e microrganismos termófilos isolados do substrato de cultivo do cogumelo. Araraquara: UNESP/IQ, 1999. 83p. (Dissertação - Mestrado)

12.http://nzfungi.landcareresearch.co.nz/html/data.asp?ID=&NAMEPKey=4487 sept.07,2008

13.Bresinsky et al. 1977, Hilber 1982, Moser 1983, Watling & Gregory 1989.

14.Stamets, P., 2005. "Notes on Nutritional Properties of Culinary-Medicinal Mushrooms", International Journal of Medicinal Mushrooms, vol. 7: 103–110.

15.Wikipedia: mushroom sept.07,2008

16.www.mushroomcouncil.org 2200 sept. 2008

17.Food Chemistry21

Volume 88, Issue 3, December 2004, Pages 425-428 Abstract

18.Food Chemistry22

Volume 73, Issue 3, May 2001, Pages 321-325Abstract

19.Hong, J.S.; Kim, Y.H.; Kim, M.K.; Kim, Y.S. (Chonbuk National Univ., Chonju (Korea R.). Dept. of Food Science and Technology); Sohn, H.S. (Chonbuk National Univ., Chonju (Korea R.). Dept. of Home Economics Education) (Feb 1989)

20.http://www.fao.org/docrep/007/y5489e/y5489e12.htm#P3401_301942 Edible mushrooms by country sept.2008

21.Fasidi, Isola O.; Ekuere, Usukama U. 1993. Studies on Pleurotus tuber-regium (Fries) Singer: cultivation, proximate composition and mineral contents of sclerotia. Food Chemistry 48: 255-258

22.Fasidi, Isola O.; Ekuere, Usukama U. 1995. Studies on Pleurotus tuber-regium (Fries) Singer: cultivation, proximate composition and mineral contents of sclerotia.

23.http://www.fao.org/docrep/007/y5489e/y5489e00.htm#TopOfPage Boa, E (2004) Wild Edible Fungi: a global overview of their uses and importance. FAO, Rome.

24. Marmion, V J et. al. The Death of Claudius, The Royal Society of Medicine, © 2002

25.Rubel, William. Amanita Muscaria

26."Trichoderma spp., including T. harzianum, T. viride, T. koningii, T. hamatum and other spp. Deuteromycetes, Moniliales (asexual classification system)". Biological Control: A Guide to Natural Enemies in North America. Retrieved on 2007-07-10.

27.Arora, David. Mushrooms Demystified, 2nd ed. Ten Speed Press

28.V.P. Gerasimenya, O.V. Efremenkova, O.V. Kamzolkina, T.A. Bogush, I.V. Tolstych, V.A. Zenkova, Antimicrobial and antitoxical action of edible and medicinal mushroom Pleurotus ostreatus (Jacq.: Fr.) Kumm. extracts, Int. J. Med. Mush. 4 (2002) 48–54.

29.L. Xia, K.T. Chu, T.B. Ng, A low-molecular mass ribonuclease from the brown oyster mushroom, J. Pept. Res. 66 (2005) 1–8.

30.K. Okamoto, S. Narayama, A. Katsuo, I. Shigematsu, H.Yanase, Biosynthesis of p-anisaldehyde by the white-rot basidiomycete Pleurotus ostreatus, J. Biosci. Bioeng. 93 (2002) 207–210.

31.K. Periasamy, Novel antibacterial compounds obtained from some edible mushrooms, Int. J. Med. Mush. 7 (2005) 443–444.

32.H. Wang, T.B. Ng, Eryngin, a novel antifungal peptide from fruiting bodies of the edible mushroom Pleurotus eryngii, Peptides, 25 (2004) 1–5.

33.C.R. Brandt, F. Piraino, Mushroom antivirals, Recent Res. Dev. Antimicrob. Agents Chemother. 4 (2000) 11–26.

34.Acharya K, Yonzone P, Rai M, Rupa A (2005). Antioxidant and nitric oxide synthase activation properties of Ganoderma applanatum. Indian. J. Exp. Biol. 43: 926–929.

35.Akpata ES, Akinrinmisi EO (1977). Antibacterial acticity of extracts of some African chewing sticks. Hos Surg, 44: 717-722.

36.Alade PI, Irobi ON (1993). Antimicrobial activities of crude extract of Acalypha wilkesiana. J. Erthnopharmacol. 39: 171-174.

37.Bauer AW, Kirby WMM, Sherris JC, Truck M (1966). Antibiotic suscep-tibility testing by a standar single dose method. Am. J. Clin Pathol. 45: 493-496.

38.Brand-Williams W, Cuvelier ME, Berset C (1995). Use of a free radical method to evaluate antioxidant activity. Lebensm. Wiss. Technol 28: 25–30.

39.Bobek P. Galbavy S (1999). Hypocholesteremic and antiatherogenic effect of oyster mushroom (Pleurotus ostreatus) in rabbits Nahrung 43: 339–342.

40.Cano A, Hernandez-Ruiz J, Garcia-Carnovas F, Acosta M, Arnao MB (1998). An end-point method for estimation of the total antioxidant activity in plant material. Phytochem. Anal. 9: 196–202.

41.Cao G, Sofic E, Prior RL (1996). Antioxidant capacity of tea and common vegetables. J. Agric. Food. Chem, 44: 3426–3431.

42.Chang ST, Miles PG (1992). Mushrooms biology—a new discipline Mycologist 6: 64–5.

43.This document is an output from a project funded by the UK Department for International Development (DFID) and the European Commission (EC) for the benefit of developing countries. The views expressed are not necessarily those of DFID or the EC.

44.Cowan MM (1999). Plant products as antimicrobial agents. Clin. Microbiol. Rev. 12: 564–582.

45.Cowan ST, Steele KJ (1974). Manual for the identification of medical bacteria.2nd edition. Cowan. Cambridge University Press.

46.Wang, H.X. Wang, T.B. Ng, A peptide with HIV-1 reverse transcriptase inhibitory activity from the medicinal mushroom Russula paludosa, Peptides, 28 (2007) 560–565.

47.N. Gunde-Cimerman, A. Plemenitas, Hypocholesterolemic activity of the genus Pleurotus (Jacq.: Fr.) P. Kumm. (Agaricaless. l., Basidiomycetes), Int. J. Med. Mush. 3 (2001) 395–397.

48.M. Sano, K. Yoshino, T. Matsuzawa, T. Ikekawa, Inhibitory effects of edible higher basidiomycetes mushroom extracts on mouse type IV allergy, Int. J. Med. Mush. 4 (2002) 37–41.

49.N. Jose, K.K. Janardhanan, Antioxidant and antitumour activity of Pleurotus florida, Curr. Sci. 79 (2000) 941–943.

50.BUNYARD, B.A.; CHAICHUCHOTE, S.; NICHOLSON, M.S.; ROYSE, D.J. Ribosomal DNA analysis for resolution of genotypic classes of Pleurotus. Mycological Research, v.2, p.143-150, 1996.

51.I.G. Meerovich, M. Yang, P. Jiang, R.M. Hoffman, V.P. Gerasimenya, A.E. Orlov, A.P. Savitsky, V.O. Popov, Study of action of cyclophosphamide and extract of mycelium of Pleurotus ostreatus in vivo on mice, bearing melanoma B16-F0-GFP, Proceedings of the SPIE, Vol. 5704, Genetically Engineered and Optical Probes for Biomedical Applications III, San Hose, California, USA (2005) pp. 214–221.

52.S.Y. Hagiwara, M. Takahashi, Y. Shen, S. Kaihou, T. Tomiyama, M. Yazawa, Y. Tamai, Y. Sin, A. Kazusaka, M. Terazawa, A phytochemical in the edible Tamogi-take mushroom (Pleurotus cornucopiae), D-mannitol, inhibits ACEactivity and lowers the blood pressure of spontaneously hypertensive rats, Biosci. Biotechnol. Biochem. 69 (2005) 1603–1605.

53.N. Jose, T.A. Ajith, K.K. Janarrdhanan, Antioxidant, anti--inflammatory, and antitumor activities of culinary-medicinal mushroom Pleurotus pulmonarius (Fr.) Quel. (Agaricomycetideae), Int. J. Med. Mush. 4 (2002) 59–66.

54.M. Zhang, L. Zhang, P.C.K. Cheung, V.E.C. Ooi, Molecular weight and anti-tumor activity of the water-soluble polysaccharides isolated by hot water and ultrasonic treatment from the sclerotia and mycelia of Pleurotus tuber-regium, Carbohydr. Polym. 56 (2004) 123–128.

55.Y.H. Gu, G. Sivam, Cytotoxic effect of oyster mushroom Pleurotus ostreatus on human androgen-independent prostate cancer PC-3 cells, J. Med. Food. 9 (2006) 196–204.

56.S.P. Wasser, Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides, Appl. Microbiol. Biotechnol. 60 (2002) 258–274.

57.M. Zhang, S.W. Cui, P.C.K. Cheung, Q. Wang, Antitumor polysaccharides from mushrooms: A review on their isolation process, structural characteristics and antitumor activity, Trends Food Sci. Technol. 18 (2007) 4–19

58.P.C. Cheung, M.Y. Lee, Fractionation and characterization of mushroom dietary fiber (nonstarch polysaccharides) as potential nutraceuticals from sclerotia of Pleurotus tuber-regium (Fries) singer, J. Agric. Food Chem. 48 (2000) 3148–3151.

59.D. Chenghua, Y. Xiangliang, G. Xiaoman, W. Yan, Z. Jingyan, X. Huibi, A beta-D-glucan from the sclerotia of Pleurotus tuber-regium (Fr.) Sing, Carbohydr. Res. 328 (2000) 629–633.

60.L. Zhang, M. Zhang, J. Dong, J. Guo, Y. Song, P.C.K. Cheung, Chemical structure and chain conformation of the water insoluble glucan isolated from Pleurotus tuber-regium, Biopolymers, 59 (2001) 457–464.

61.F.R. Rosado, E.R. Carbonero, C. Kemmelmeier, C.A. Tischer, P.A.J. Gorin, M. Iacomini, A partially 3-O-methylated (1,4)-linked a-D-galactan and a-D-mannan from Pleurotus ostreatoroseus Sing, FEMS Microbiol. Lett. 212 (2002) 261–265.

62.F.R. Rosado, E.R. Carbonero, R.F. Claudino, C.A. Tischer, C. Kemmelmeier, M. Iacomini, The presence of partially 3-O-methylated mannogalactan from the fruit bodies of edible basidiomycetes Pleurotus ostreatus 'florida' Berk. and Pleurotus ostreatoroseus Sing., FEMS Microbiol. Lett. 221 (2003) 119–124.

63.M. Zhang, L. Zhang, P.C. Cheung, J. Dong, Fractionation and characterization of a polysaccharide from the sclerotia of Pleurotus tuber-regium by preparative size-exclusion chromatography, J. Biochem. Biophys. Methods, 56 (2003) 281–289.

64.M. Pramanik, S. Mondal, I. Chakraborty, D. Rout, S.S. Islam, Structural investigation of a polysaccharide (Fr. II) isolated from the aqueous extract of an edible mushroom, Pleurotus sajor-caju, Carbohydr. Res. 340 (2005) 629–636.

65.D. Rout, S. Mondal, I. Chakraborty, M. Pramanik, S.S. Islam, Chemical analysis of a new (1,3)-, (1,6)-branched glucan from an edible mushroom, Pleurotus florida, Carbohydr. Res. 340 (2005) 2533–2539.

66.D. Rout, S. Mondal, I. Chakraborty, S.S. Islam, The structure of a polysaccharide from Fraction-II of an edible mushroom, Pleurotus florida, Carbohydr. Res. 341 (2006) 995–1002.

67.E.R. Carbonero, A.H.P. Gracher, F.R. Smiderle, F.R. Rosado, G.L. Sassaki, P.A.J. Gorin, M. Lacomini, A b-glucan from the fruit bodies of edible mushrooms Pleurotus eryngii and Pleurotus ostreatoroseus, Carbohydr. Polym. 66 (2006) 252–257.

68.Y. Tao, L. Zhang, Determination of molecular size and shape of hyperbranched polysaccharide in solution, Biopolymers, 83 (2006) 414–423.

69.M. Zhang, P.C. Cheung, L. Zhang, Evaluation of mushroom dietary fiber (nonstarch polysaccharides) from sclerotia of Pleurotus tuber-regium (Fries) Singer as a potential antitumor agent, J. Agric. Food Chem. 49 (2001) 5059–5062.

70.M. Zhang, L. Zhang, P.C. Cheung, Molecular mass and chain conformation of carboxymethylated derivatives of beta-glucan from sclerotia of Pleurotus tuber-regium, Biopolymers, 68 (2003) 150–159.

71.M.E.I. Zhang, P.C.K. Cheung, L. Zhang, C.M. Chiu, V.E.C. Ooi, Carboxymethylated b-glucans from mushroom sclerotium of Pleurotus tuber-regium as novel water-soluble anti-tumor agent, Carbohydr. Polym. 57 (2004) 319–325.

72.Y. Tao, L. Zhang, P.C. Cheung, Physicochemical properties and antitumor activities of water-soluble native and sulfated hyperbranched mushroom polysaccharides, Carbohydr. Res. 341 (2006) 2261–2269.

73.M. Zhang, P.C.K. Cheung, L.C.M. Chiu, E.Y.L. Wong, V.E.C. Ooi, Cell-cycle arrest and apoptosis induction in human breast carcinoma MCF-7 cells by carboxymethylated [beta]-glucan from the mushroom sclerotia of Pleurotustuber-regium, Carbohydr. Polym. 66 (2006) 455–462.

74.R.G. Mahajan, S.I. Patil, D.R.K. Mohan, P. Shastry, Pleurotus eous mushroom lectin (PEL) with mixed carbohydrate inhibition and antiproliferative activity on tumor cell lines, J. Biochem. Mol. Biol. Biophys. 6 (2002) 341–345.

75.P.H. Ngai, T.B. Ng, A ribonuclease with antimicrobial, antimitogenic and antiproliferative activities from the edible mushroom Pleurotus sajor-caju, Peptides, 25 (2004) 11–17.

76.P. Ngai, T. Ng, A hemolysin from the mushroom Pleurotus eryngii, Appl. Microbiol. Biotechnol. 72 (2006) 1185–1191.

77.V. Shlyakhovenko, V. Kosak, S. Olishevsky, Application of DNA from mushroom Pleurotus ostreatus for cancer biotherapy: A pilot study, Experim. Oncol. 28 (2006) 132–135.

78.H. Wang, J. Gao, T.B. Ng, A new lectin with highly potent antihepatoma and antisarcoma activities from the oyster mushroom Pleurotus ostreatus, Biochem. Biophys. Res. Commun. 275 (2000) 810–816.

79.P. Bobek, S. Galbavy, Effect of pleuran (beta-glucan from Pleurotus ostreatus) on the antioxidant status of the organism and on dimethylhydrazine-induced precancerous lesions in rat colon, Brit. J. Biomed. Sci. 58 (2001) 164–168.

80.D. Kamilya, D. Ghosh, S. Bandyopadhyay, B.C. Mal, T.K. Maiti, In vitro effects of bovine lactoferrin, mushroom glucan and Abrus agglutinin on Indian major carp, catla (Catla catla) head kidney leukocytes, Aquaculture, 253(2006) 130–139.

81.I. Sarangi, D. Ghosh, S.K. Bhutia, S.K. Mallick, T.K. Maiti, Anti-tumor and immunomodulating effects of Pleurotus ostreatus mycelia-derived proteoglycans, Int. Immunopharmacol. 6 (2006) 1287–1297

82.J.C. Ho, S.C. Sze, W.Z. Shen, W.K. Liu, Mitogenic activity of edible mushroom lectins, Biochim. Biophys. Acta, 1671 (2004) 9–17

83.J.H. Yang, H.C. Lin, J.L. Mau, Antioxidant properties of several commercial mushrooms, Food Chem. 77 (2002) 229–235.

84.Y.I. Shi, A.E. James, I.F.F. Benzie, J.A. Buswell, Mushroom-derived preparation in the prevention of H2O2-induced oxidative damage to cellular DNA, Teratogen. Carcin. Mut.22 (2002) 103–111.

85Y.L. Lee, G.W. Huang, Z.C. Liang, J.L. Mau, Antioxidant properties of three extracts from Pleurotus citrinopileatus, Lebensm. Wiss. Technol. 40 (2007) 823–833.

86.S.H. Lo, Quality evaluation of Agaricus bisporus, Pleurotus eryngii, Pleurotus ferulae and Pleurotus ostreatus and their antioxidant properties during postharvest storage, MSc Thesis, National Chung-Hsing University, Taichung, Taiwan (2005).

87.S.H. Hu, Z.C. Liang, Y.C. Chia, J.L. Lien, K.S. Chen, M.Y. Lee, J.C. Wang, Antihyperlipidemic and antioxidant effects of extracts from Pleurotus citrinopileatus, J. Agric. Food Chem. 54 (2006) 2103–2110.

88.L. Li, T.B. Ng, M. Song, F. Yuan, Z.K. Liu, C.L. Wang, Y. Jiang, M. Fu, F. Liu, A polysaccharide–peptide complex from abalone mushroom (Pleurotus abalonus) fruiting bodies increases activities and gene expression of antioxidant enzymesand reduces lipid peroxidation in senescence-accelerated mice, Appl. Microbiol. Biotechnol. 75 (2007) 863–869.

89.N. Jose, T.A. Ajith, K.K. Janardhanan, Methanol extract of the oyster mushroom, Pleurotus florida, inhibits inflammation and platelet aggregation, Phytother. Res. 18 (2004) 43–46.

90.Kawagishi H, Ryuichi RI, Kanao T, Keishiro TM, Hitoshi S, Hagiwara IT, et al. ( 1989;) Fractionation and antitumor activity of the water-insoluble residue of Agaricus blazei fruiting bodies. Carbohyd Res 186:: 267–73[CrossRef][ISI][Medline]

91.Ohno N, Furukawa M, Miura NN, Adachi Y, Motoi M, Yadomae TI. ( 2001;) Antitumor-glucan from the cultured fruit body of A. blazei. Biol Pharm Bull 24:: 820–8.[CrossRef][ISI][Medline].

92.Ohno N, Hayashi M, Iino K, Suzuki I, Oikawa S, Sato K, et al. ( 1986;) Effect of glucans on the antitumor activity of grifolan. Chem Pharm Bull 34:: 2149–54.

93.Dong Q, Yao J, Yang X. ( 2002;) Structural characterization of water-soluble of ß-D-glucan from fruiting bodies of Agaricus blazei Murr. Carbohyd Res 337:: 1417–21.[CrossRef][ISI][Medline].

94.Mizuno T, Hagiwara T, Nakamura T, Ito H, Shimura K, Sumiya T. ( 1990;) Antitumor activity and some properties of water-soluble polysaccharides from "Himematsutake", the fruiting body of Agaricus blazei Murril. Agric Biol Chem 54:: 2889–96.

95.Camelini CM, Maraschin M, de Mendonca MM, Zucco C, Ferreira AG, Tavares LA. ( 2005;) Structural characterization of ß-glucans of Agaricus brasiliensis in different stages of fruiting body maturity and their use in nutraceutical products. Biotechnol Lett 27:: 1295–9.[CrossRef][ISI][Medline].

96.Mizuno M, Morimoto M, Minato K, Tsuchida H. ( 1998;) Polysaccharides from Agaricus blazei stimulate lymphocyte T-cell subsets in mice. Biosci Biotechnol Biochem 62:: 434–7.[CrossRef][Medline]

97.Mizuno M, Minato K, Ito H, Kawade M, Terai H, Tsuchida H. ( 1999;) Antitumor polysaccharide from the mycelium of liquid-cultured Agaricus blazei mill. Biochem Mol Biol Int 47:: 707–14.[ISI][Medline]

98.Sorimachi K, Akimoto K, Inafuku K, Okubo A, Yamazaki S. ( 2001;) Secretion of TNF-, IL-8 and nitric oxide by macrophages activated with Agaricus blazei Murril fractions in vitro. Cell Struct Funct 26:: 103–8.[CrossRef][ISI][Medline]

99.Cooper EL. ( 2004;) Commentary on CAM and NK Cells by Kazuyoshi Takeda and Ko Okumura. eCAM 1:: 29–34

100.Chil. Chem. Soc. Vol.53 no.2 Conception June 2008.

101.Agarwl ,1994

102.IJC-Indian J. Chem., 1979,17B, 87,88,180,297,412,417.

103.IJC-Indian J. Chem. Soc., 180, 57, 417, 761.

104.Egon Stahl, Thin layer chromatograohy, Springer Berlin, Heide Berg Newyork, 369, 1969.

105.Pavia, D.L. Gray M. Lamman Grey S. Kinz (1976).

106.Silverstein. R. M., 1981, Spectrometric Identifications of Organic Compounds, Jhon Wiley & Sons, fourth edition, 194-195, 268.

107.SofowanaA,1993 Medicinal plants and traditional medicine in Africa

108.Harbon,J.B.,1973 Phytocimal methods .49-188

109.Trease G.E.and Evans W.C.,1989, pharmascopy 11th edn.

110.Boham. B.A. and Kocipai,A.C. 1974, Flavonoids and condenced tannins from leaves of Hawaiian Vaccinium and V.calcycinium. Pacific SCI. 48,458-463

111.Cocks,L.V. and Rede,C.V., 1966.65.

112.Sarwar G.,Masud-un-nabi, M.A.Rahman A. Jabbar Mian, 1991, free and bound fatty acids: a comparison of Dhabdhabey S-718 jute with Corchorus capsaularis and Corchorus oletorious,j. Bangladesh Acad. Sci.15(2). 133-138

113.Lambert et al. Spectroscopy of Organic Chemistry. 1987

114.Pavia,D.L., Lampman, G.L. and Kriz, G.S., Introduction to Spectroscopy, 2001. 3rd edition USA

115.Pharmaceutical Journal VOL-4>No.2>December 2005

116.World Intellectual Property Organization