CHEMICAL CONSTITUENTS OF *GONIOTHALAMUS TAPISOIDES*

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DISSERTATION SUBMITTED IN FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF SCIENCE

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ABSTRACT

Goniothalamus tapisoides Mat Salleh, a plant from Annonaceae family, has been studied. Collected from Sarawak, the bark of this plant were dried and grounded before phytochemical study was performed. The chromatographic separation on the dichloromethane extracts of the bark gave eleven compounds – six known and five new. Six known compounds were identified as goniothalamin **1**, pterodondiol **164**, liriodenine **114**, 9-deoxygoniopypyrone **15**, benzamide **161** and cinnamic acid **155**. Five new compounds are goniomicin A **157**, goniomicin B **158**, goniomicin C **159**, goniomicin D **160** and tapisoidin **162**.

ABSTRAK

Goniothalamus tapisoides Mat Salleh, sejenis tumbuhan dari famili Annonaceae, telah dikaji. Dikumpulkan dari Sarawak, bahagian kulit pokok tumbuhan ini terlebih dahulu dikering dan dikisarkan sebelum kajian fitokimia dijalankan. Pemisahan secara kromatografi pada ekstrak diklorometana untuk bahagian kulit telah memencilkan sebelas sebatian – enam dikenali dan lima baru. Enam sebatian yang dikenali telah dipencilkan daripada bahagian kulit telah dikenalpasti sebagai goniotalamin **1**, pterodondiol **164**, liriodenina **114**, 9-deoxygoniopypyron **15**, benzamida **161** and asid sinamik **155**. Lima sebatian yang baru adalah goniomicin A **157**, goniomicin B **158**, goniomicin C **159**, goniomicin D **160** dan tapisoidin **162**.

ACKNOWLEDGEMENTS

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3. Mr. Leong for his help to run bioactivity test.
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ABBREVIATIONS

α	Alpha
Å	Armstrong
β	Beta
γ	Gamma
br s	Broad singlet
CC	Column chromatography
$CDCl_3$	Deuterated chloroform
CH_3	Methyl group
cm^{-1}	Per centimeter
COSY	H-H correlation spectroscopy
δ	Chemical shift
DEPT	Distortioness Enchancement by Polarisation Transfer
db	Double bond
dd	Doublet of doublets
ddd	Doublet of doublet of doublet
dt	Doublet of triplet
er	*erythro*
ε	Molar absorptivity
Ed_{50}	Effective doss of 50% activity
FT-NMR	Fourier Transform-Nuclear Magnetic Resonance

1H	Proton NMR
g	Gram
GCMS	Gas Chromatography Mass Spectrometry
HMBC	Heteronuclear Chemical Shift Correlation
HPLC	High Performance Liquid Chromatography
Hz	Hertz
IC_{50}	Concentration required to inhibit of 50% activity
IR	Infrared
J	Coupling constant (Hz)
L	Litre
λ	Lambda (maximum wavelength)
m	Metre
m	Multiplet
m/z	Mass to charge ratio
MeOH	Methanol
MHz	Mega Hertz
MS	Mass spectrum
ml	Mililitre
μg/ml	Microgram per mililitre
nm	Nanometer
NMR	Nuclear Magnetic Resonance
NOE	Nuclear Overhauser Effect
OCH_3	Methoxyl group
OCH_2O	Methylenedioxy group
OH	Hydroxyl group
ppm	Parts per million
q	Quartet
quin	Quintet
s	Singlet
t	Triplet
th	*threo*
TLC	Thin Layer Chromatography
UV	Ultraviolet
^{13}C	Carbon-13 NMR
2D NMR	Two dimensional NMR

CHAPTER 1

INTRODUCTION

1.1 Introduction

The term natural products today quite commonly refer to herbs, herbal concoctions, dietary supplements, traditional Chinese medicine, or alternative medicine.[1] In fact, natural products are usually either of prebiotic origin or originate from microbes, plants or animals sources which are secondary metabolites with molecular weight less than 2000 amu produced by a living organism that are not strictly necessary for the survival of the organism. Secondary metabolites are produce in response to needs and challenges of the natural environment.[2]

Nature has been a source of therapeutic agents since early human history and an impressive number of modern drugs have been derived from natural sources, which many of it based on their use in traditional medicine. Over the last century, a number of top selling drugs have been developed from natural products for example vincristine from *Vinca rosea*, morphine from *Papaver somniferum* and Taxol from *Taxus brevifolia*.

In recent years, a significant revival of interest in natural products as a potential source for new medicines has been observed among academia as well as pharmaceutical companies.[2] According to Cragg *et al*, 39% of 520 new approved drugs between 1983 and 1994 were natural products or their derivatives, and 60-80% of antibacterial and anticancer drugs were from natural origins. In 2000, approximately 60% of all drugs in clinical trials for the multiplicity of cancers had natural origins.[2]

Secondary metabolites keep evolving as nature are continually carrying out its own version of combinational chemistry for the over 3 billion years during which bacteria have inhabited the earth. For the useful metabolites, the biosynthetic genes were retained, and genetic modifications further improved the process. Combinatorial chemistry occurred by nature is much more sophisticated than in the laboratory.

Therefore, a great amount and different variety of natural products have been found naturally. The total numbers of natural products have been estimated to be over 500,000 in the world. Until today, there are about 160,000 natural products have been identified and this value is estimated increasing by 10,000 per year. [3]

The earliest medicinal plant research in Malaysia was on phytochemical survey of plants in Malaysia that was carried out by Arthur in 1954 and later the screening of 200 species for Peninsular Malaysia for the presence of alkaloids were carried out. [4] These two publications marked the beginning of medicinal plant research in Malaysia.

Malaysia has about 12,000 species of flowering plants of which about 1,300 are said to be medicinal and only about a hundred have been investigated fully for their potential.[5] Due to the medicinal importance of natural products, the author has embarked the study of the chemical constituents from the stem bark of *Goniothalamus tapisoides* that belongs to the family of Annonaceae. This genus is known to produce compounds with cytotoxic activity such as goniothalamin.[17] In this study, chemical constituents will be isolated and structurally elucidated using various chromatographic and spectroscopic techniques. To the knowledge of the author the chemical constituents of this plant has never been reported.

The objectives of the project are threefold. First, to isolate chemical constituents from species *Goniothalamus tapisoides* by using chromatographic methods. The chromatographic methods we used are column chromatography, thin layer chromatography (TLC) and flash column. Second, structural elucidation on the components which is carried out by NMR and mass spectroscopy (MS). And finally, cytotoxic studies on several cell lines (lung, prostate, skin, pancreatic, liver, colon and breast) will be carried out on the isolated compounds.

1.2 Annonaceae: Distribution and Habitat[5,6,7,8]

The Annonaceae (custard-apple family) is the most diverse family of primitive angiosperms with about 200 genera and 2500 species. It is suggested that the early diversity centre of Annonaceae is in the north part of West Gondwanaland. Thus, the family Annonaceae has come into existence since late Cretaceous.

Annonaceae is a pantropical family that well developed in tropical regions mainly at low elevations in moist forests. Except for two related North American genera (*Asimina* and *Deeringothamnus*), generally they are distributed over the tropical areas of America, Africa and Asia.

According to Takhtajan, 30 genera and 740 species are found in America continent. While 40 genera and 450 species are found in Africa and Madagascar. These show that the genus have better diversity in America continent compared to Africa and Madagascar. While in Asia alone, about 60 genera and 1000 species can be found. Within Asia area, Indo-Malaysia has greatest concentration of genera and species compared to others area.

Annonaceae is a family of woody, resinous plants, comprising only trees, shrubs and climbers, whose fruits are, bunches of big-seeded berries for the detection of such animals as squirrels, monkeys and bats. Annonaceae is commonly known by Malays as 'Pisang-Pisang' or variants of this on account of the bunch of carpels suggesting bananas.

The plants of this order are very important economically, pharmacologically and nutritionally. The seeds of the Annonaceae plants can produce edible oil and soap. The wood can be used to manufacture alcohol and the fragrant flowers like *Cananga odorata*, are important as raw material for the perfumery industry.

1.3 Annonaceae : General Appearance and Morphology[9,10,11,12]

The trees of Annonaceae are shrubs, erect or climbing. They may reach more than 4 meters high and the shrub may go to about 30 cm high. The bark usually smooth and entire, pale grey or buff to brown. The twigs are pubescent or tomentose, but rarely glabrous. Young twigs become glabrous sooner or later.

The leaves are always simples, alternate and entire without stipules and membranous or coriceous. The base may be acute, rounded, emiginate, ordate or unequal sided. The apex is acute, acuminate or less often obtuse.

The flowers are usually solitary and accented. The scented flowers are very famous and the most popular species with fragrant flowers is *Cananga ordorata* (Ylang-ylang) when the flower is not solitary, the influorescense is often a few flower cyme, usually condensed. The petals have a wonderful diversity and are of the greatest diagnostic value in Annonaceae.

The stamens are normally numerous, arranged in spirals on a convex or slightly flattened torus. The apex may be oblique, truncate, flat topped, two lobed with a little depression in the middle, convex, conical or produced into long point.

The ovaries are usually oblong, cylindric, terete or angled and occasionally slightly falcate. They are usually covered with a pubescent or tomentose indumentums. The style may be present or absent. When present it is short as in *Monocarpia* and *Popowia* or elongated and slender as in *Xylopia* and *Goniothalamus*. In all genera the stigma is split or slightly grooved on the top.

The fruits of Annonaceae are very important role in determining the genera. In fact, Annonaceae are subdivided into two subfamilies, *Annonaideae* and

Monodoroideae on the basis of the fruit alone. In *Annonaideae* a great majority has apocarpous carpels. They are stalked or sessile whereas in a few generathe carpels are united into a many called syncarp with erect stigmas. While in *Monodoroideae*, the carpels are united into a one celled ovary with placentation and radiating stigmas.

Classification and determination of a genus is dependent on a combination of characters, for instance that of the petal and the fruit. There are 38 genera, 198 native and 5 cultivated species besides 17 varieties of Annonaceae in Penisular Malaysia. Scheme 1 and Table 1 illustrate the summarized classification made by Sinclair.

Scheme 1.1: Classification of Annonaceae

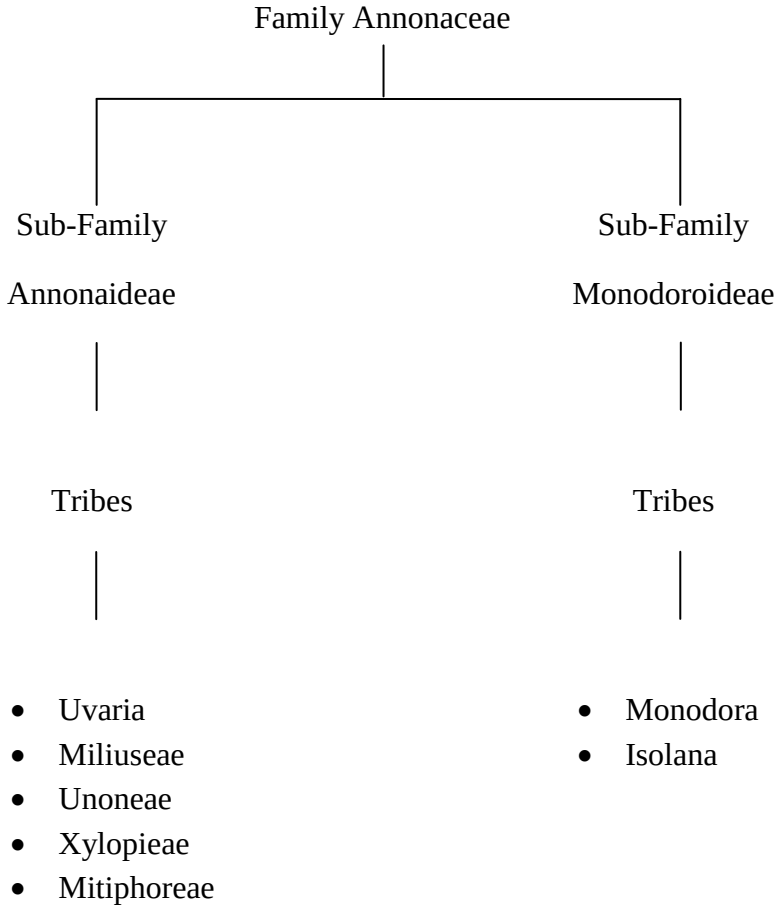


Table 1.1: Genera of Annonaceae

Tribes	**Genera**
Uvarieaea	*Sageraea* *Stelechocarpus* *Kingstonia* *Enicosanthum* *Trivalvaria* *Uvaria* *Cyathostemma* *Rauwenhoffia* *Ellipeia*
Unoneae	*Cyathocalyx* *Artobotrys* *Desmos* *Monocarpia* *Oncodostigma* *Polyalthia* *Cananga* *Mezzettia* *Disepalum* *Meiogyne*
Xylopeae	*Xylopia* *Anaxagorea* *Fissistigma* *Pyramidanthe* *Mitrella* *Melodorum*
Miliuseae	*Marsipopetalum* *Phaeanthus* *Miliusa* *Alphonsea* *Platymitro* *Orophea*
Mitiphoreae	*Pseuduvaria* *Neo-Uvaria* *Goniothalamus* *Oxymitra* *Mitrephora* *Popowia*
Annonineae	*Annona*

1.4 Genus - *Goniothalamus*[13]

Goniothalamus is one of the largest genera of paleotropical Annonaceae with over 120 species distributed throughout the tropics and subtropics with some species being used widely as traditional medicines. The trees of genus *Goniothalamus* are normally shrubs and small trees. The leaves are leathery or papery. The nerves are prominent with ladder-like reticulations or indistinct with lax network of reticulations. While for the flowers, they are usually axillary, sometimes terminal or cauliflorous.

For more specific, the petals reveal in the form of valvate, quite leathery and outer larger than inner. This genus also has many stamens with quite linear-oblong, connectives apiculate and flat topped or convex. The ovaries are numerous, cylindrical, pubescent or glabrous with the style of linear and grooved on the anterior side. The stigmas are more or less funnel shaped with two lobed, rarely cylindrical and truncate. The fruits are stalked or sessile with 1-2 seeds.

In regards to the pharmacological potentials of *Goniothalamus* species, there is evidence to suggest that this taxon has the ability to elaborate series of acetogenins and styryl lactones which are cytotoxic against a broad array of cancer cells including breast, colon, kidney and pancreatic carcinoma cells.

1.5 Botanical Aspect of *Goniothalamus tapisoides* Mat Salleh[14]

G. tapisoides Mat Salleh is known as ‘selada’ by the Malays or ‘semukau’ by the Iban. It is a small tree around 5 m in height. It is endemic to Borneo, especially the sourthern part of Sarawak.

1.6 Traditional Medicinal Uses of *Goniothalamus* species

Plant from *Goniothalamus* species have been used as folk medicine in several countries such as Malaysia, Indonesia, Taiwan and Philppines.[13] Table 1.2 lists the medicinal uses of some *Goniothalamus* species on the basis of reference 13.

Table 1.2: The medicinal uses of *Goniothalamus* species

***Goniothalamus* species & Localities**		**Part of Plant**	**Treatments**
1.	*G. amuyon* Peninsular Malaysia, Taiwan and Philippines	Seeds Fruits	– In Taiwan, the seeds are used to treat scabies. – In Philippines, the seeds are evoked with oil make an effective liniment to treat rheumatism. – And also decoctions of seeds are used to treat tymparites. – In Philippines, the fruits are used to treat stomachic.
2.	*G. dolichocharpus* Peninsular Malaysia	Roots	– The roots are boiled and taken orally by the Kelabit community to ease stomachache.
3.	*G. giganteus* Peninsular Malaysia	Roots Leaves	– The roots are used to abort and treat colds. – The heated leaves are applied to swellings.
4.	*G. macrophyllus* Peninsular Malaysia and Jawa, Indonesia	Leaves Roots	– The leaves are used to abrogate fever. – The burnt leaves also been noted to be fragrant and are an effective mosquito repellent. – The decoction of roots is given as a postpartum remedy and to cause abortion.

***Goniothalamus* species & Localities**		**Part of Plant**	**Treatments**
5.	*G. scortechinii* Peninsular Malaysia		– A decoction of this species alone or in mixture is given as a postpartum protective medicine. – In Malay, it is used to improve blood circulation.
6.	*G. tapis* Peninsular Malaysia, Borneo and Indonesia	Roots Bark	– The roots are used an abortifacient during early months of pregnancy. – In Java, Indonesia, an infusion of the roots is used to treat typhoid fever. – In Indonesia, the bark is used as mosquito repellent.

CHAPTER 2

GENERAL CHEMICAL ASPECTS

2.1 General[15]

The chemical compositions of a plant cannot be defined precisely for a given tree or even tree of same species. Chemical composition varies with tree part, type of plant geographic location, climate and soil conditions. However generally, plant of the same species shows same chemotaxonomic similarities.

Chemicals from plants can be broadly classified as primary and secondary compounds or metabolites. The primary metabolites are in all cells and play a central role in metabolism and reproduction of those cells. These compounds include the nucleic acids, common amino acids and sugars. Most primary metabolites exert their biological effect within the cell or organism that is responsible for their production. Secondary metabolites have a restricted distribution and are often characteristic of individual genera, species or strains and they are formed along specialized pathways from primary metabolites. They are non-essential to life.

For a long time there was no clear role of secondary metabolites and they were often described as waste products of the plant's metabolism. Today we consider them as the means by which the plant interacts with other organisms in the immediate environment. Therefore secondary metabolites have been attracted interest because of their biological effect on other organisms.

Drugs, coloring matters, essential oils, flavoring substances are example of natural products obtained from the forest. Secondary metabolites are the minor component of plants. They are termed as extractives since they do not form part of the cell wall structure and can often be extracted by means of suitable organic solvents and water without destroying the structure of the plant tissues.

The chemical examination of secondary compounds has been a major aspect in the development of organic chemistry. Their chemical structures are complex and diverse and cover almost the whole spectrum of organic chemicals. Alkaloids, terpenoids, styryl-lactones, acetogenins, tannins, resins, flavonoids and glycosides are the examples of secondary compounds. Many of these compounds have marked physiological properties and some have been or are important substances in industries and medicine. The biologically active constituents of medicinal, commercial and poisonous plants have been studied and it have been estimated that over 40% of medicines have their origins in these natural products.

Styryl-lactones and acetogenins are two major types of bioactive compounds isolated from *Goniothalamus* species. Other types of compounds also found in *Goniothalamus* species are alkaloids, terpenes and flavonoids. Interestingly, both styryl-lactones and acetogenins are completely different in terms of chemical structures but their cellular activities are involving the mitochondria in mammals. In this study, author had been aiming for styryl-lactones compounds and discussed briefly the general chemical aspects of other interesting chemical constituents of *Goniothalamus* plants.

2.2 Styryl-lactones[15,16]

Styryl-lactones are a group of secondary metabolites isolated mainly from various species of shrubs and trees. The styryl-lactones are found primarily in the *Goniothalamus* species (Annonaceae) that have demonstrated to posses interesting biological properties. Styryl-lactones have been reported to posses cytotoxic, anti-tumor, pesticidal, teratogenic and embryotoxic activities.

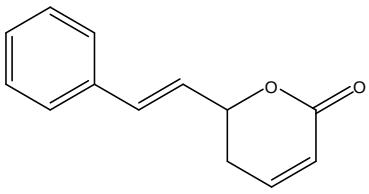
Styryl-lactones are low molecular weight phenolic compounds which have basic skeleton of 13 carbon atoms that includes (as the name styryl-lactone implies) a styryl

or pseudo-styryl fragment linked to a lactone moiety. Styryl-lactones are cytotoxic secondary metabolites having γ-, δ-, or ζ-lactone rings. Over 30 different styryl-lactones have been isolated and described from various species of *Goniothalamus*.

Styryl-lactones can be classified into six groups based on the structural characteristics of the skeletons. These groups are; styryl-pyrones, furano-pyrones, furano-furones, pyrano-pyrones, butenolides , and heptolides.

2.2.1 Styryl-pyrones[17]

Goniothalamin **1**, goniodiol **2** and etharvendiol **3** are some members of this group.



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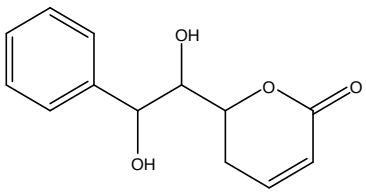

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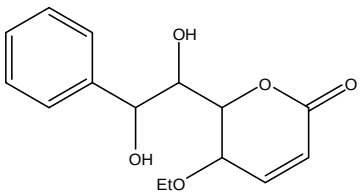
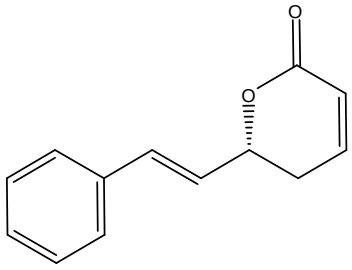


3

Goniothalamin **1** was firstly isolated from the dried bark of *Cryptocarya caloneura* in the year 1967. Later it was isolated from *Cryptocarya moschata*, and various species of *Goniothalamus*. Goniothalamin **1** is the first styryl-lactone found in Annonaceae, it shows a potent mosquito larvicide, weak bacterial and significant antifungal activity against a wide range of gram-positive and gram-negative bacteria and fungi.

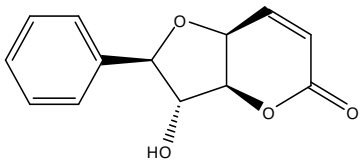


4

(*R*)-Goniothalamin **4** has displayed in vitro cytotoxic effect especially by inducing apoptosis on different cancer cell lines [cervical carcinoma (Hela); gastric carcinoma (HGC-27); breast carcinoma (MCF-7, T47D, MDA-MB-231); leukemia (HL-60), ovarian carcinoma (Caov-3)]. Interestingly, this effect was shown to be selective for cancer cell lines with no significant cytotoxicity toward non-malignant cells.

2.2.2 Furano-pyrones

The furano-pyrone skeleton represents the second most abundant class of styryl lactones in *Goniothalamus*. Altholactone **5**, also called goniothalenol, is the first member in this group that was first identified from *Polyalthia* and eight years later was isolated from several species of *Goniothalamus*. Others example of this group are isoaltholactone **6**, 2-*epi*-altholactone **7**, goniofupyrone **8**, goniotharvensin **9**, and etharvensin **10**.



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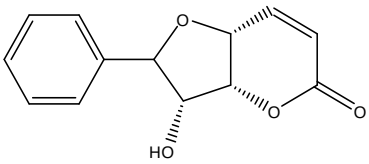

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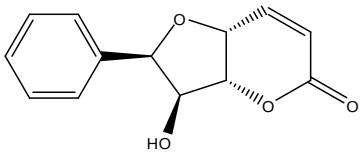

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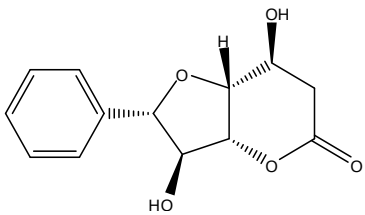

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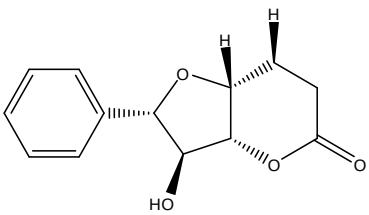

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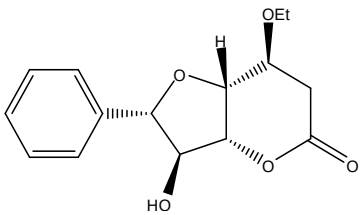
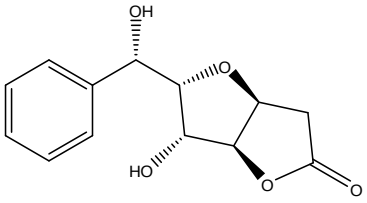


10

2.2.3 Furano-furones[18]

Goniofufurone **11** and 7-epi-goniofufurone **12** are members of this group. They are isolated from the stem bark of *G. giganteus*. Goniofufurone **11** has cytotoxic activity against A-549 (human lung carcinoma), MCF-7 (human breast adenocarcinoma) and HT-29 (human colon adenocarcinoma) cell lines with $ED_{50} < 4$ μg/ml.



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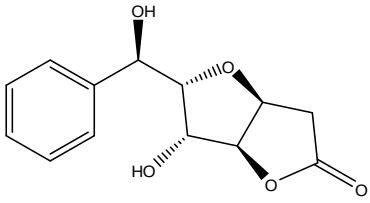
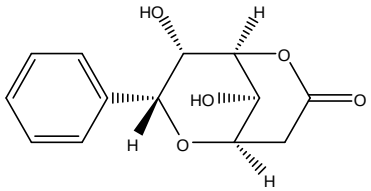


12

2.2.4 Pyrano-pyrones[19]

The examples of pyrano-pyrone styryl lactones are goniopypyrone **13**, leiocarpin-A **14** and 9-deoxygoniopypyrone **15**. They are also exhibiting non-selective activity against human tumour cell lines.



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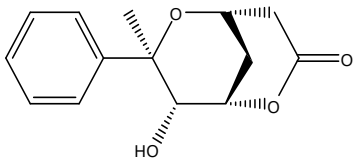

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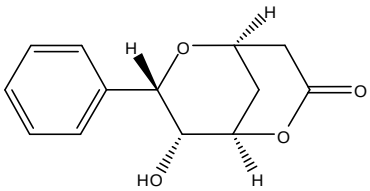
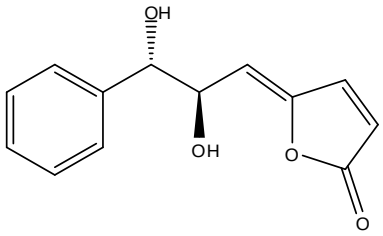


15

2.2.5 Butenolides[20]

Two well known compounds in this group are goniobutenolide-A **16** and goniobutenolide-B **17**. They were isolated from *G. giganteus*. Biological activity of goniobutenolide-A **16** was tested against four different tumour cell lines (HL-60, HCT-

8, MDA/MB-435 and SF295) and it has no effect on cell lines HL-60, MDA/MB-435 and SF295, but for the cell line HCT-8 a modest cytotoxicity was observed (IC_{50} = 101.5 μM).



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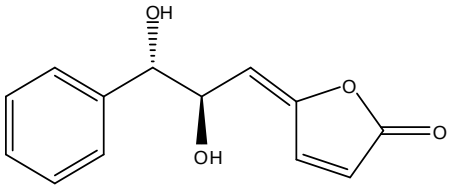
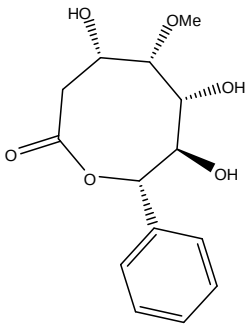


17

2.2.6 Heptolides[21]

In this group, gonioheptolides-A **18** and gonioheptolides-B **19** were isolated from the stem bark of *G. giganteus*. Almuheptolides-A **20** and Almuheptolides-B **21** were isolated from the stem bark of *G. arvensis*. Compounds of this group contain a saturated eight-membered lactone moiety.



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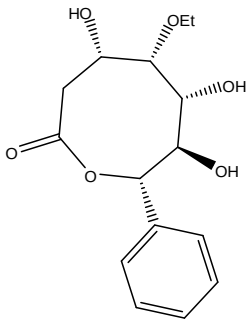

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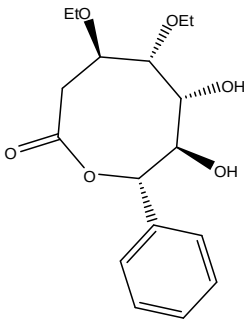

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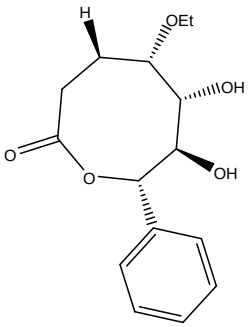


21

2.3 Acetogenins[22,23,24]

Acetogenins are naturally occurring polyketides which have so far only been characterized from members of the family Annonaceae including in the genus *Goniothalamus* particularly, *G. giganteus*, *G. donnaiensis*, and *G. gardenri*. Uvaricin

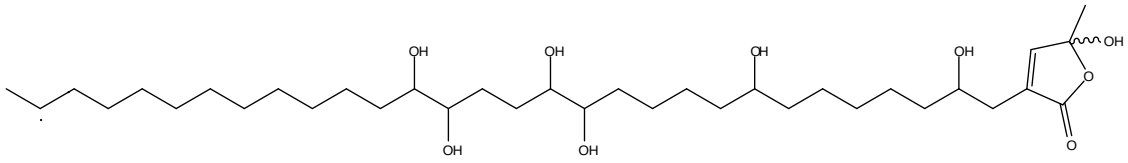
was first discovered by Jolad in 1982, and then scientists have been isolated more than 400 acetogenins in the past 25 years from Annonaceae family. The general skeleton of acetogenins is unbranched C_{35}-C_{37} fatty acid, terminated with a 2,4-disubstituted-γ-lactone (sometimes rearranged to a 2,4-disubstituted ketolactone) moiety. Several oxygenated functions, such as hydroxyl, ketone, epoxide, tetrahydrofuran (THF) and tetrahydropyran (THP) may be present, as well as double and triple bonds. Thus several types of acetogenins have been characterized based on the nature of functional groups which are present.

Structurally, most of these acetogenins may be classified into six major groups, i.e. non-tetrahydrofuran (THF), mono-THF, adjacent bis-THF, non-adjacent bis-THF, tri-THF and tetrahydropyran (THP) acetogenins. They can be sub grouped again according to the form of the γ-lactone ring (unsaturated γ-methyl-γ-lactone, a propanone substituted unsaturated γ-lactone or a β-hydroxy-γ-methyl-γ-lactone).

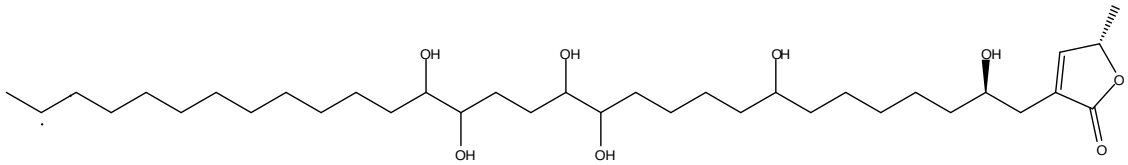
All acetogenins contains multiple stereocenters, the elucidation of which often presents stereochemical problems. Acetogenins do not form crystals suitable for X-ray crystallographic analysis due to their waxy nature. Acetogenins are very potent inhibitors of the NADH-ubiquinone reductase (Complex I) activity of mammalian mitochondria. As very potent mitochondrial inhibitors, the acetogenins are a class of promising anticancer, anti-infective, and pesticidal natural compounds.

2.3.1 Non-tetrahydrofuran[25]

Acetogenins within this group is linear. The examples of non-THF are donhepocin **22**, 34-epi-donhepocin **22'**, donhexocin **23** and donbutocin **24**. They were isolated from *G. donnaiensis*; **22** and **22'** containing rare γ-hydroxymethyl-γ-lactone that isolated as an epimeric pair.



22(22')



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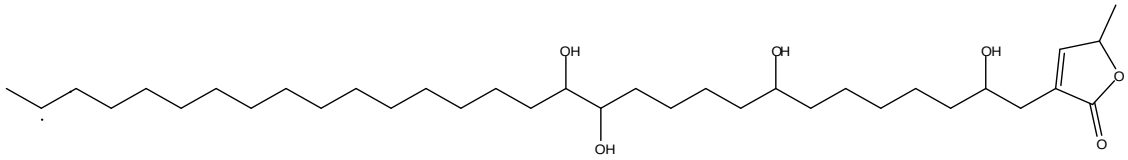
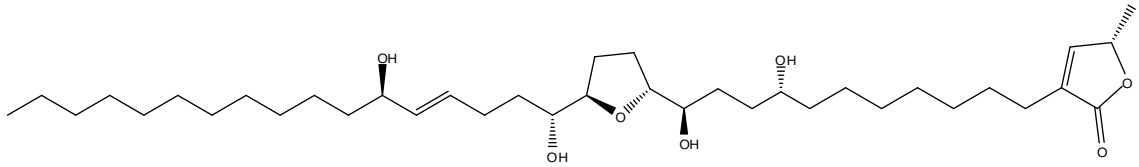


24

2.3.2 Mono-tetrahydrofuran[26]

Gigantransenins A **25**, B **26** and C **27** are three examples of mono-THF acetogenins that isolated from the bark of *G. giganteus*. Gigantransenins A, B and C are the first examples of acetogenins having *trans* double bonds. They also showed selective inhibitory effects on the human breast tumor cell-line (MCF-7) comparable with the potency of adriamycin.



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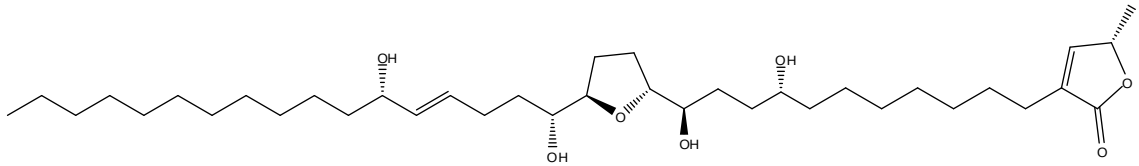

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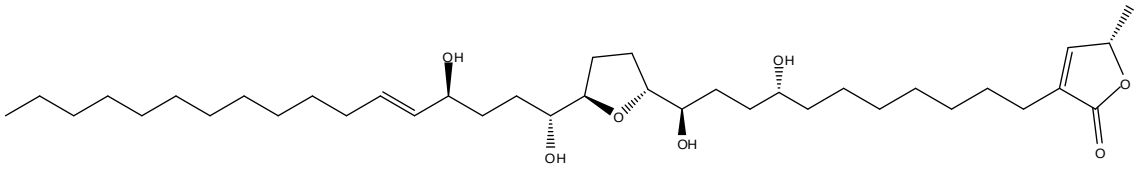
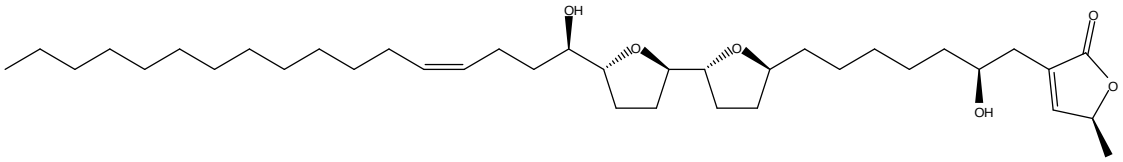


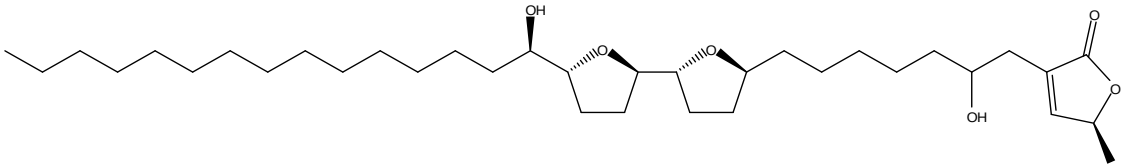
27

2.3.3 Adjacent bis-tetrahydrofuran

Goniodenin **28**, asimilobin **29** and longimicin C **30** are examples of adjacent bis-THF that isolated from *G. giganteus*.



28



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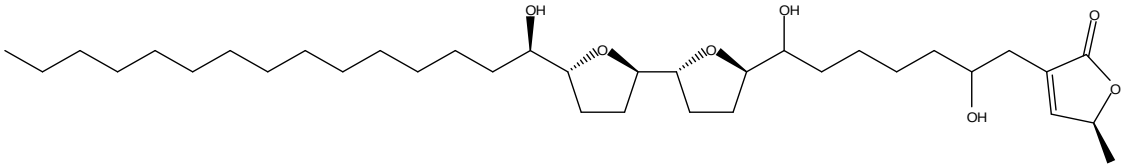
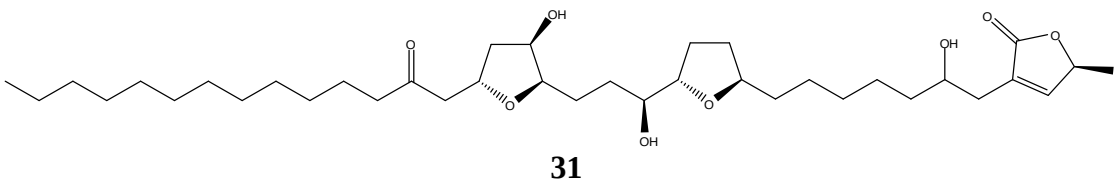


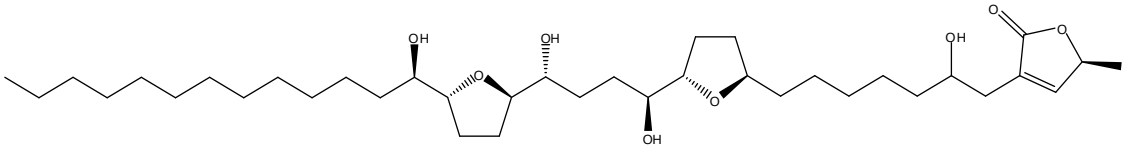
30

2.3.4 Non-adjacent bis-tetrahydrofuran

Goniotriocin **31**, gigantecin **32** and 4-deoxygigantecin **33** are some members of this group. They are also isolated from *G. giganteus*.



31



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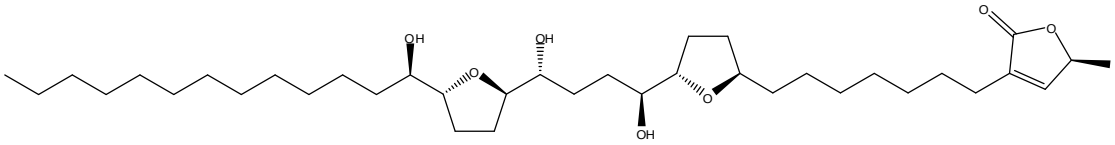
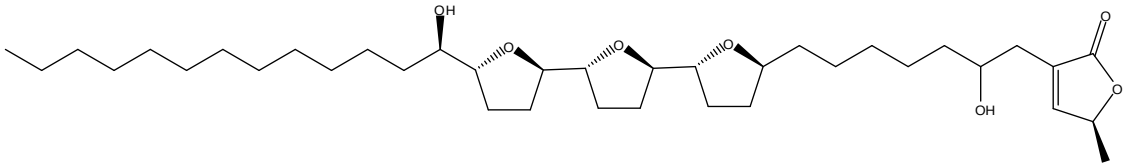


33

2.3.5 Tri-tetrahydrofuran

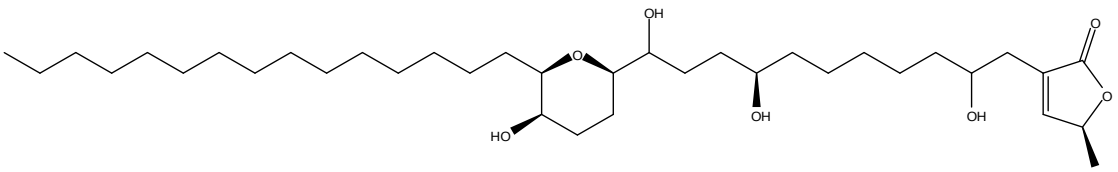
Goniocin **34** is the first compounds having tri-tetrahydrofuran (THF) moiety that had been isolated from *G. giganteus*. Until now there are the only tri-THF had been isolated from this genus.



34

2.3.6 Tetrahydropyran[27]

Pyranicin **35** and pyragonicin **36** are the first mono-THP acetogenins. Both pyranicin and pyragonicin exhibited a selective cytotoxic against the pancreatic cell line (PACA-2) in a panel of six human solid tumor cell lines, with pyranicin showing ten times the potency of adriamycin.



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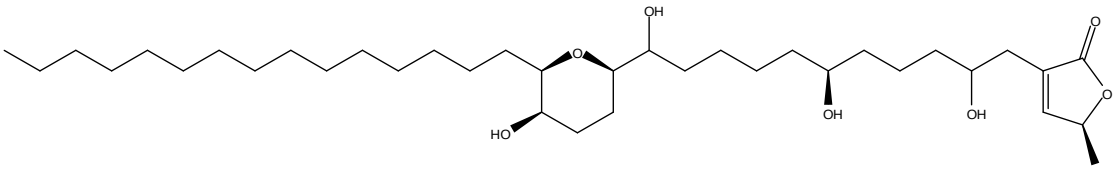


36

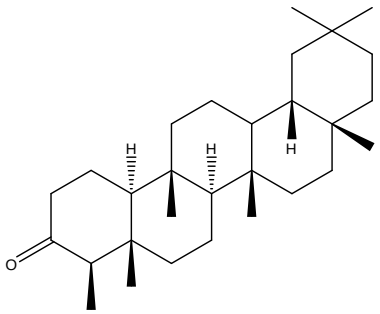
2.4 Terpenes[28]

Terpenes are natural lipid products that are built up from isoprene units. An isoprene unit is a five-carbon unit with the connectivity of the molecule isoprene. Terpenes are classified by the number of isoprene units that they contain (Table 2.1). Terpenes are responsible for many of the flavour, fragrances, and colours of plants. Some are plant hormones, pheromones, poisons, or drugs.

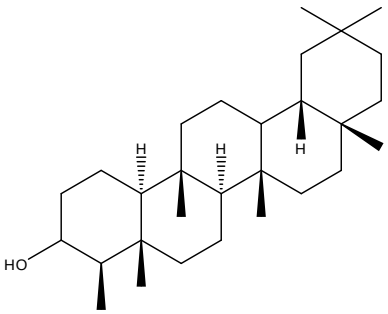
Table 2.1: Common Classification of Terpene Groups

Group	No. of carbon	Isoprene unit
Monoterpene	10	2
Sesquiterpene	15	3
Diterpene	20	4
Sesterterpene	25	5
Triterpene	30	6
Tetraterpene	40	8

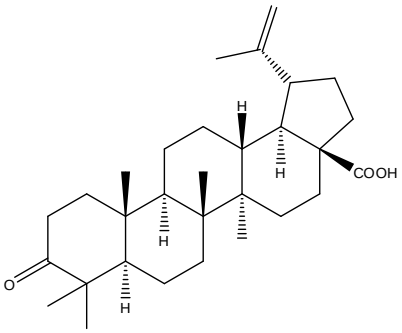
Friedelin **37**, friedelinol **38** and betullinic acid **39** are examples of triterpene that isolated from *G. thwaitessi*.



37



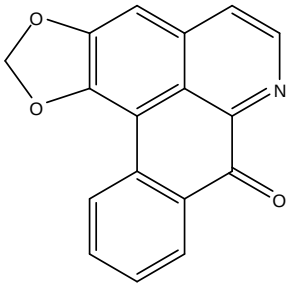
38



39

2.5 Alkaloids

Several classes of compounds such as azaanthraquinones, aristolactams, aporphines and amino-napthoquinones types of alkaloids have been reported in this genus. For example there are five alkaloids isolated from the stems of *G. amuyon*; liriodenine **40**, griffithazanone A **41**, griffithazanone B **42**, velutinam **43** and cepharanone B (aristolactam BII) **44**.



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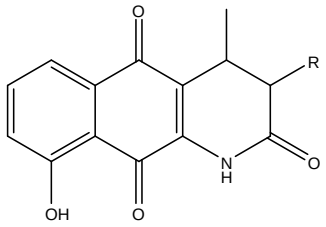
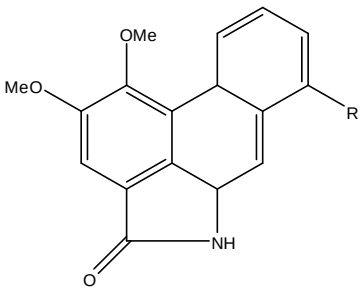


41 R=OH
42 R=H

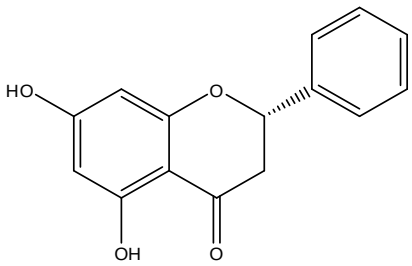


43 R=OH
44 R=H

2.6 Flavonoids

Flavonoids are polyphenolic compounds possessing 15 carbon skeleton arranged in C_6-C_3-C_6 fashion. In biological roles, flavonoids are important plant pigments for flower colouration producing yellow or red/blue pigmentation in petals designed to attract pollinator animals.

Several flavonoid compounds have been found in *Goniothalamus* species. Among them is pinocembrine **45**, it has been isolated from *G. borneensis*, *G. giganteus*, *G. laoticus* and *G. macrophyllus*.



45

Many *Goniothalamus* species have been studied before. Table 2.2 lists all types of compounds isolated from *Goniothalamus* species.

Table 2.2: Chemical Constituents from *Goniothalamus* species

Species and Compounds	Type	Reference
G. amuyon		
7-Acetylgoniodiol **54**	Styryl-pyrone	29
8-Acetylgoniodiol **55**	Styryl-pyrone	30
8-Chlorogoniodiol **58**	Styryl-pyrone	31
9-Deoxygoniopypyrone **15**	Pyrano-pyrone	32
7,8-*diepimer*-Goniotriol **62**	Styryl-pyrone	30
Goniotriol **60**	Styryl-pyrone	30
Goniothalamin **1**	Styryl-pyrone	31
Goniothalamin oxide **49**	Styryl-pyrone	31
8-Methoxygoniodiol **57**	Styryl-pyrone	31
Annonacin **73**	Mono-THF	33
Corossolin **76**	Mono-THF	33
Gigantriocin **84**	Mono-THF	33
Cepharanone B **44**	Alkaloid	34
Griffithazanone A **41**	Alkaloid	34
Griffithazanone B **42**	Alkaloid	34
Liriodenine **40**	Alkaloid	30
Velutinam **43**	Alkaloid	34
G. andersonii		
Iso-5-deoxygoniopypyrone **68**	Pyrano-pyrone	35
Goniodiol **2**	Styryl-pyrone	35
Goniothalamin **1**	Styryl-pyrone	35
Goniothalamin oxide **49**	Styryl-pyrone	35
G. arvensis		
3-Acetylaltholactone **64**	Furano-pyrone	36
5-Acetoxyisogoniothalamin oxide **50**	Styryl-pyrone	36
Almuheptolide A **20**	Heptolide	21
Almuheptolide B **21**	Heptolide	21
Altholactone **5**	Furano-pyrone	37
2-*epi*-Altholactone **7**	Furano-pyrone	38
Arvensin **65**	Furano-pyrone	38
Etharvendiol **3**	Styryl-pyrone	39
Etharvensin **10**	Furano-pyrone	40
Garvensintriol **63**	Styryl-pyrone	21
Goniofufurone **11**	Furano-pyrone	21
Goniotharvensin **9**	Furano-pyrone	37
Isoaltholactone **6**	Furano-pyrone	37
G. borneensis		
Goniobutenolide A **16**	Butenolide	41
Goniobutenolide B **17**	Butenolide	41
Goniofufurone **11**	Furano-furone	41
Goniothalamin **1**	Styryl-pyrone	41
Goniothalenol **5**	Furano-pyrone	41

Species and Compounds	Type	Reference
Goniotriol **60**	Styryl-pyrone	41
Aristolactam A-III **134**	Alkaloid	42
Goniothalactam **136**	Alkaloid	41
Pinocembrine **45**	Flavanoid	43
G. cardiopetalus		
Altholactone **5**	Furano-pyrone	44
Cardiopetalolactone **66**	Furano-pyrone	44
Goniopypyrone **13**	Pyrano-pyrone	44
Cardiobutanolide **70**	Butenolide	45
Goniothalamin **1**	Styryl-pyrone	45
Goniodiol **2**	Styryl-pyrone	45
Goniofufurone **11**	Furano-furone	45
Goniofupyrone **8**	Furano-pyrone	45
G. cheliensis		
Cheliensisin A **51**	Styryl-pyrone	46
Goniothalamin **1**	Styryl-pyrone	47
Cheliensisin B **74**	Mono-THF	47
Cheliensisin C **75**	Mono-THF	47
Goniodiol **2**	Styryl-pyrone	48
Isoaltholactone **6**	Furano-pyrone	48
Leiocarpin A **14**	Pyrano-pyrone	48
Pinocembrine **45**	Flavonoid	48
(3S)-2-oxo-5,12-dimethoxy-methylbenz[*f*]indoline **125**	Alkaloid	49
G. dolichocarpus		
9-Deoxyisogoniopypyrone **68**	Pyrano-pyrone	50
Goniodiol **2**	Styryl-pyrone	50
Goniothalamin oxide **49**	Styryl-pyrone	50
Goniothalamin **1**	Styryl-pyrone	50
5-*β*-Hydroxygoniothalamin **47**	Styryl-pyrone	51
Annonacin **73**	Mono-THF	50
G. donnaiensis		
Annonacin **73**	Mono-THF	52
Donbutocin **24**	Linear	53
Donhepocin **22**	Linear	53
34-*epi*-Donhepocin **22'**	Linear	53
Donhexocin **23**	Linear	54
Donnaienin **77**	Mono-THF	55
Donnaienin A **78**	Mono-THF	52
34-*epi*-Donnaienin A	Mono-THF	52
Donnaienin B **79**	Mono-THF	52
34-*epi*-Donnaienin B	Mono-THF	52
Donnaienin C **93**	Mono-THF	56
34-*epi*-Donnaienin C	Mono-THF	56

Species and Compounds	Type	Reference
Donnaienin D **118**	Linear	56
34-*epi*-Donnaienin D	Linear	56
2,4-*cis*-Gigantetrocinone **99**	Mono-THF	53
2,4-*trans*-Gigantetrocinone	Mono-THF	53
Goniodonin **85**	Mono-THF	53
Goniothalamicin **77**	Mono-THF	52
Isoannonacin **80**	Mono-THF	52
Murisolin **79**	Mono-THF	52
G. fulvus		
Goniothalamin **1**	Styryl-pyrone	57
G. gardneri		
Annonacin **73**	Mono-THF	58
Isoannonacin **80**	Mono-THF	58
Gardnerilin A **116**	Linear	59
Gardnerilin B **117**	Linear	59
Gardnerin **80**	Mono-THF	58
Gardnerinin **81**	Mono-THF	60
34-*epi*-Gardnerinin	Mono-THF	60
Gigantetrocin A **82**	Mono-THF	58
Gigantetrocin B **83**	Mono-THF	58
Goniothalamicin **77**	Mono-THF	58
Goniothalamusin **119**	Linear	61
2′,4′-dihydroxy-4,6-Dimethoxychalcone **141**	Chalcone	62
2′,4′-dihydroxy-4,6′-dimethoxydihydrochalcone **138**	Chalcone	62
2′-hydroxy-4,4′,6′-trimethoxydihydrochalcone **137**	Chalcone	62
4, 2′,4′-trihydroxy-6′-methoxydihydrochalcone **139**	Chalcone	62
(rel)-1β, 2α-di-(2,4-dihydroxy-6-methoxybenzoyl)-3β, 4α-di-(4-methoxyphenyl)-cyclobutane **142**	Dihydrochalcone	62
Annulatin **146**	Flavonoid	62
Flavokawain A **140**	Chalcone	62
Mearnsitrin **145**	Flavonoid	62
Naringenin trimethyl ether **143**	Flavonoid	62
Tsugafolin **144**	Flavonoid	62
G. giganteus		
8-Acetylgoniotriol **61**	Styryl-pyrone	63
Altholactone **5**	Furano-pyrone	64
Goniobutenolide A **16**	Butenolide	65
Goniobutenolide B **17**	Butenolide	65
Goniodiol **2**	Styryl-pyrone	18
Goniofufurone **11**	Furano-furone	63

Species and Compounds	**Type**	**Reference**
7-*epi*-goniofufurone **12**	Furano-furone	18
Goniofupyrone **8**	Furano-pyrone	65
Gonioheptolide A **18**	Heptolide	66
Gonioheptolide B **19**	Heptolide	66
Goniopypyrone **13**	Pyrano-pyrone	63
9-Deoxygoniopypyrone **15**	Pyrano-pyrone	18
Goniothalamin **1**	Styryl-pyrone	64
Goniotriol **60**	Styryl-pyrone	67
Annomontacin **91**	Mono-THF	68
4-Deoxyannomontacin **92**	Mono-THF	69
cis-Annomontacinone **107**	Mono-THF	69
trans-Annomontacinone	Mono-THF	69
Annonacin **73**	Mono-THF	70
2,4-*cis*-Isoannonacin **110**	Mono-THF	71
2,4-*trans*-Isoannonacin	Mono-THF	71
Asimilobin **29**	Adjacent bis-THF	72
Giganenin **94**	Mono-THF	73
Giganin **115**	Linear	66
Gigantecin **32**	Non Adjacent bis-THF	74
4-Deoxygigantecin **33**	Non Adjacent bis-THF	73
2,4-*cis*-Gigantecinone **114**	Non Adjacent bis-THF	71
2,4-*trans*-Gigantecinone	Non Adjacent bis-THF	71
Gigantetrocin **82**	Mono-THF	18
4-Acetylgigantetrocin A **95**	Mono-THF	75
2,4-*cis*-Gigantetrocinone **108**	Mono-THF	69
2,4-*trans*-Gigantetrocinone	Mono-THF	69
Gigantetronenin **96**	Mono-THF	68
Gigantetronin **97**	Mono-THF	68
Gigantransenin A **25**	Mono-THF	26
Gigantransenin B **26**	Mono-THF	26
Gigantransenin C **27**	Mono-THF	26
Gigantriocin **84**	Mono-THF	18
Gigantrionenin **101**	Mono-THF	68
cis-Gigantrionenin **102**	Mono-THF	75
Goniocin **34**	Tri-THF	76
Goniodenin **28**	Adjacent bis-THF	72
Gonionenin **103**	Mono-THF	77
2,4-*cis*-gonioneninone **109**	Mono-THF	78
2,4-*trans*-gonioneninone	Mono-THF	78
Goniotetracin **104**	Mono-THF	78
Goniothalamicin **86**	Mono-THF	70
Goniotriocin **31**	Non Adjacent bis-THF	79
Goniotrionin **87**	Mono-THF	78
Longicoricin **105**	Mono-THF	69
Longifolicin **90**	Mono-THF	69
Longimicin C **30**	Adjacent bis-THF	71
Pyragonicin **36**	Pyran	27

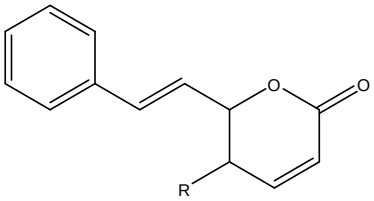
Species and Compounds	Type	Reference
Pyranicin **35**	Pyran	27
Squamocin **120**	Adjacent bis-THF	76
Xylomaticin **106**	Mono-THF	69
2,4-*cis*-Xylomaticinone **111**	Mono-THF	79
2,4-*trans*-Xylomaticinone	Mono-THF	79
Pinocembrin **45**	Flavonoid	64
G. grandiflorus		
Isoaltholactone **6**	Furano-pyrone	80
G. griffithii		
8-Acetylgoniotriol **61**	Styryl-pyrone	81
8-Acetylgoniofufurone **67**	Furano-furone	82
8-Acetylgoniopypyrone **69**	Pyrano-pyrone	82
7-Acetylgoniodiol **54**	Styryl-pyrone	82
Altholactone **5**	Furano-pyrone	81
9-Deoxygoniopypyrone **15**	Pyrano-pyrone	81
Goniodiol **2**	Styryl-pyrone	82
Goniodiol diacetate **56**	Styryl-pyrone	82
Goniothalamin **1**	Styryl-pyrone	81
Goniotharvensin **9**	Furano-pyrone	81
Goniofufurone **11**	Furano-furone	81
Goniopypyrone **13**	Pyrano-pyrone	82
Goniotriol **60**	Styryl-pyrone	82
Isoaltholactone **6**	Furano-pyrone	82
Aristolactam A-II **134**	Alkaloid	83
Griffithdione **121**	Alkaloid	83
Griffithinam **135**	Alkaloid	83
Griffithazanone A **41**	Alkaloid	83
Griffithazanone B **42**	Alkaloid	83
Taliscanine **133**	Alkaloid	83
Velutinam **43**	Alkaloid	83
G. howii		
Goniothalamin **1**	Styryl-pyrone	84
Howiinin A **52**	Styryl-pyrone	85
Howiicin A (Annonacin) **73**	Mono-THF	84
Howiicin B **88**	Mono-THF	84
Howiicin C **76**	Mono-THF	84
Howiicin D **84**	Mono-THF	86
Howiicin E **89**	Mono-THF	86
Howiicin F **82**	Mono-THF	86
Howiicin G **83**	Mono-THF	86
G. laoticus		
2-*epi*-altholactone **7**	Furano-pyrone	87
Altholactone **5**	Furano-pyrone	87
3-Acetylaltholactone **64**	Furano-pyrone	88

Species and Compounds	Type	Reference
9-Deoxygoniopypyrone **15**	Pyrano-pyrone	88
Goniotriol **60**	Styryl-pyrone	88
Goniofufurone **11**	Furano-furone	87
Goniopypyrone **13**	Pyrano-pyrone	87
Howiinin A **52**	Styryl-pyrone	88
Pinocembrine **45**	Flavonoid	87
5-hydroxy-3-amino-2-aceto-1,4-naphthoquinone **124**	Alkaloid	87
Nordicentrine **123**	Alkaloid	88
Cinnamic acid **155**		88
β-sitosterol **156**		88
G. leiocarpus		
7-*epi*-Goniodiol **53**	Styryl-pyrone	89
Goniothalamin **1**	Styryl-pyrone	90
Leiocarpin A **14**	Pyrano-pyrone	89
Leiocarpin B **71**		89
Leiocarpin C **59**	Styryl-pyrone	89
Leiocarpin E **72**		91
Annonacin **73**	Mono-THF	90
Corossolin **76**	Mono-THF	90
Gigantriocin **84**	Mono-THF	90
Murisolin **79**	Mono-THF	90
G. macrophyllus		
Goniothalamin **1**	Styryl-pyrone	92
Goniothalamin oxide **49**	Styryl-pyrone	92
Pinocembrine **45**	Flavonoid	93
G. malayanus		
Isoaltholactone **6**	Furano-pyrone	94
G. marcanii		
Marcanine A **127**	Alkaloid	95
Marcanine B **128**	Alkaloid	95
Marcanine C **129**	Alkaloid	95
Marcanine D **130**	Alkaloid	95
Marcanine E **131**	Alkaloid	95
Dielsiquinone **132**	Alkaloid	95
5-hydroxy-3-amino-2-aceto-1,4-naphthoquinone **124**	Alkaloid	95
G. montanus		
Isoaltholactone **6**	Furano-pyrone	94
G. ridleyi		
Goniothalamin **1**	Styryl-pyrone	96
Goniothalamin oxide **49**	Styryl-pyrone	96

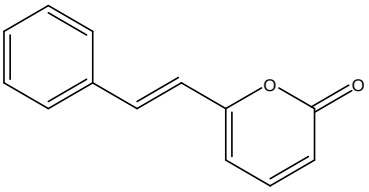
Species and Compounds	**Type**	**Reference**
Isoaltholactone **6**	Furano-pyrone	96
G. scortechinii		
Altholactone **5**	Furano-pyrone	97
Goniofufurone **11**	Furano-furone	97
Goniopypyrone **13**	Pyrano-pyrone	97
Goniothalamin **1**	Styryl-pyrone	98
Goniotriol **60**	Styryl-pyrone	97
Crytomeridiol **154**	Sesquiterpene	99
Pinocembrine **45**	Flavonoid	97
Scorazanone **126**	Alkaloid	99
G. sesquipedalis		
Goniodiol **2**	Styryl-pyrone	43
Goniodiol diacetate **56**	Styryl-pyrone	43
Goniothalamin **1**	Styryl-pyrone	100
5-Acetoxyisogoniothalamin oxide **50**	Styryl-pyrone	101
Goniotriol **60**	Styryl-pyrone	43
Gigantetrocin **82**	Mono-THF	102
G. tamirensis		
9-Deoxygoniopypyrone **15**	Pyrano-pyrone	103
8-*epi*-deoxygoniopypyrone **64**	Pyrano-pyrone	103
G. tapis		
Arvensin **65**	Furano-pyrone	104
Goniothalamin **1**	Styryl-pyrone	98
Isoaltholactone **6**	Furano-pyrone	94
G. tenuifolius		
3,5,7,3',4'-pentamethoxyflavone **148**	Flavonoid	105
5,7,3',4'-tetrahydroxy-3-methoxyflavone **149**	Flavonoid	105
4'-hydroxy-3,5,7,3'-tetramethoxyflavone **152**	Flavonoid	105
3'-hydroxy-3,5,7,4'-tetramethoxyflavone **153**	Flavonoid	105
Aristolactam A-II **134**	Alkaloid	106
Cepharanone B **44**	Alkaloid	106
Kumatakenin **144**	Flavonoid	105
Norcepharadione B **122**	Alkaloid	106
Pachypodol **151**	Flavonoid	105
Retusin **147**	Flavonoid	105
Taliscanine **133**	Alkaloid	106
Velutinam **43**	Alkaloid	106
G. thwaitessi		
Annulatin **146**	Flavonoid	62
Friedelin **37**	Triterpene	62
Friedelinol **38**	Triterpene	62

Species and Compounds	**Type**	**Reference**
Betullinic acid **39**	Triterpene	62
Mearnsitrin **145**	Flavonoid	62
G. umbrosus		
5-Acetoxygoniothalamin **46**	Styryl-pyrone	107
Dehydrogoniothalamin **48**	Styryl-pyrone	107
Goniothalamin **1**	Styryl-pyrone	107
G. uvarioides		
5-Acetylgoniothalamin **37**	Styryl-pyrone	108
Goniothalamin **1**	Styryl-pyrone	108
G. velutinus		
Altholactone **5**	Furano-pyrone	109
Goniothalamin **1**	Styryl-pyrone	109
Annonacin **73**	Mono-THF	109
Aristolactam B-II **44**	Alkaloid	109

Styryl-lactones



R= OAc **46**
R= OH **47**



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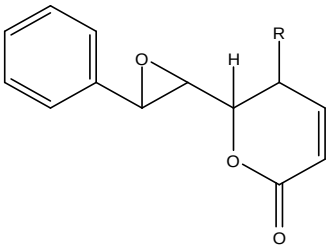
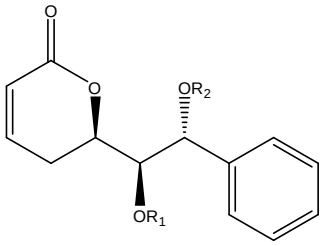
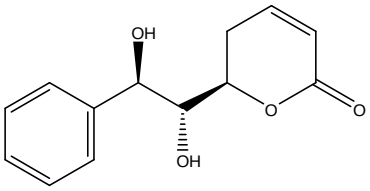


R= H **49**
R= OAc **50**
R= OAc **51**
R= O-Cinnamoyl **52**



R_1= Ac, R_2= H **53**
R_1= H, R_2= Ac **54**
R_1= Ac, R_2= Ac **55**
R_1= H, R_2= Me **56**
R_1= H, R_2= Cl **57**



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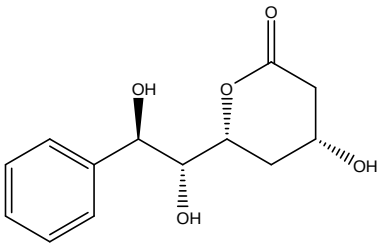

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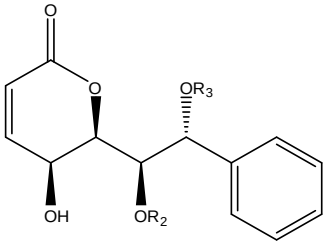
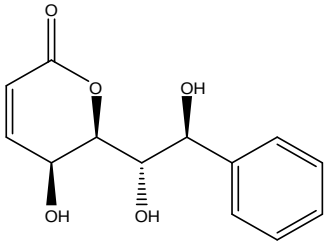


R_1= H, R_2= H **60**
R_1= H, R_2= Ac **61**



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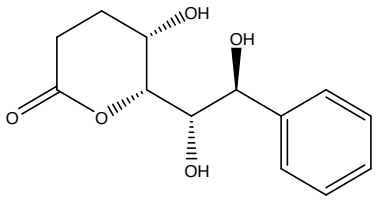

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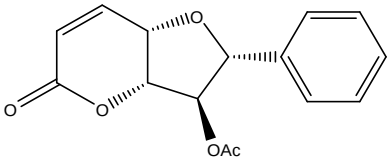

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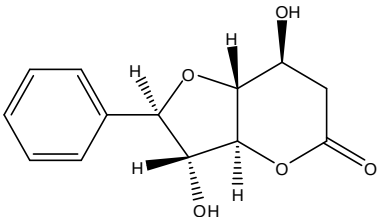

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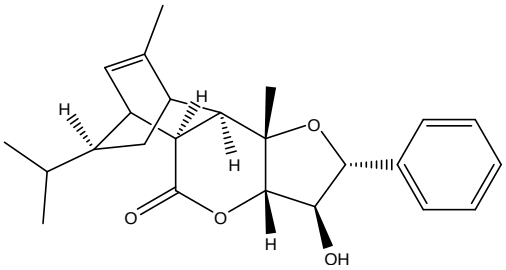

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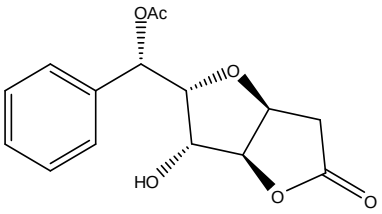

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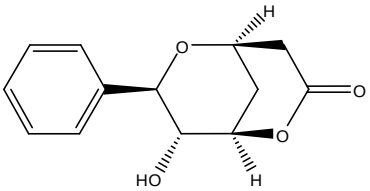

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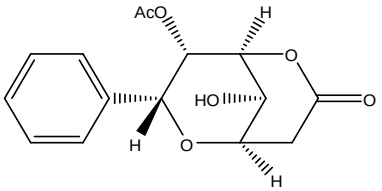

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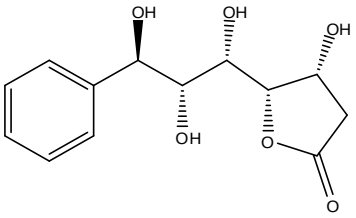

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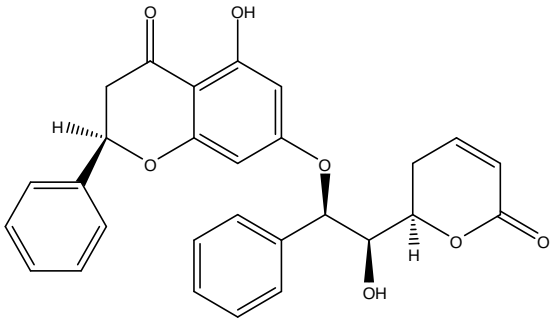

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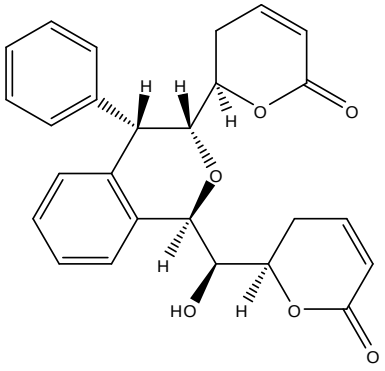


72

Acetogenins

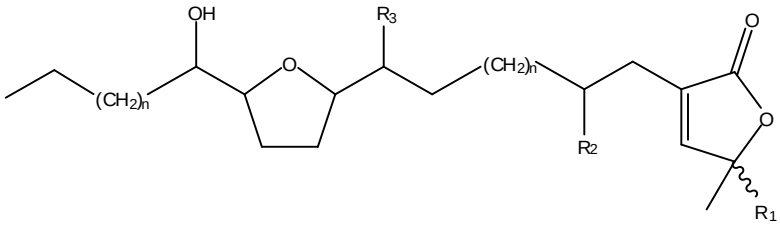


Table 2.3: Mono THF Acetogenins with 35 carbons

Compound Name	**Furan ring and Position**	**R_1**	**R_2**	**R_3**	**Others and Position**
73 Annonacin	*er/trans/er* at 15	H	OH	OH	OH at 10
74 Cheliensisin B	At 15	H	OH	OH	OH at 10 & 11, strereochemitry not stated
75 Cheliensisin C	At 15	H	OH	OH	OH at 10, strereochemitry not stated
76 4-deoxyannonacin =corossolin =howiicin C	*th/trans/th* at 15	H	H	OH	OH at 10
77 Donnaienin	*er/trans/er* at 13	H	OH	OH	OH at 10 & 15
78 Donnaienin A 34-*epi*-donnaienin A	*er/trans/er* at 15	OH	OH	OH	mixture
79 Donnaienin B 34-*epi*-donnaienin B	*trans/er* at 9	H	OH	H	OH at 17 & 18, (*er*),mixture
80 Gardnerin	*er/trans/er* at 15	H	OH	OH	OH at 8 & 10
81 Gardnerinin 34-*epi*-gardnerinin	*trans/er* at 11	OH	OH	H	OH at 10, 19 & 20 (*er*),mixture
82 Gigantetrocin =gigantetrocin A =densicomacin-2 =howiicin F	*trans/th* at 9	H	OH	H	OH at 17 & 18 (*th*)
83 Gigantetrocin B =howiicin G	*trans/th* at 9	H	OH	H	OH at 17 & 18 (*th*), isomer of gigantetrocin

84 Gigantriocin =howiicin D	*trans/th* at 9	H	H	H	OH at 17 & 18 (*th*)
85 Goniodonin	*er/trans/er* at 13	OH	OH	OH	OH at 10
86 Goniothalamicin	*th/trans/th* at 13	H	OH	OH	-
87 Goniotrionin	*th/trans/th* at 9	H	OH	H	OH at 16, *cis* db at 17-18
88 Howiicin B =murisolin	*th/trans/th* at 15	H	OH	H	-
89 Howiicin E =muricatetrocin A	At 11	H	OH	H	strereochemitry is not stated
90 Longifolicin	*th/trans/th* at 13	H	OH	OH	-

Note: *er*=*erythro* and *th*=*threo*; db=double bond

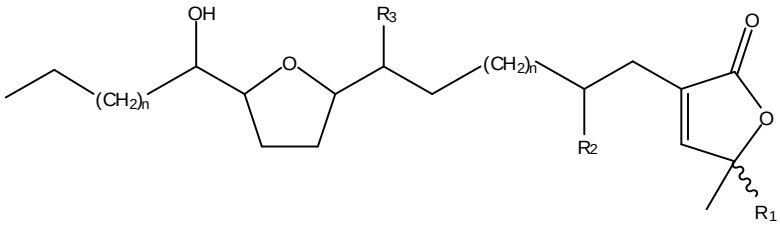


Table 2.4: Mono THF Acetogenins with 37 carbons

Compound Name	**Furan ring and Position**	**R_1**	**R_2**	**R_3**	**Others and Position**
91 Annomontacin	*er/trans/er* at 17	OH	OH	OH	OH at 10
92 4-deoxyannomontacin A	*er/trans/er* at 17	H	H	OH	OH at 10
93 Donnaienin C 34-*epi*-donnaienin C	*er/trans/er* at 15	OH	OAc	OH	OH at 10, mixture
94 Giganenin	*th/trans/th* at 13	H	H	OH	OH at 10 and db at 21-22
95 4-acetylgigantetrocin A	*trans/th* at 9	H	OAc	H	OH at 17 & 18 (*th*)
96 Gigantetronenin	*trans/er* at 9	H	OH	H	OH at 17 & 18 (*th*), *cis* db at 21-22

97 Gigantetronin	*trans/th* at 9	H	OH	H	OH at 17 & 18 (*th*)
101 Gigantrionenin	*trans/th* at 9	H	H	H	OH at 17 & 18 (*th*)
102 *cis*-gigantrionenin	*cis/th* at 9	H	H	H	OH at 17 & 18 (*th*)
103 Gonionenin	*th/trans/th* at 13	H	OH	OH	*cis*, db at 21-22
104 Goniotetracin	*th/trans/th* at 13	H	OH	OH	OH at 10
105 Longicoricin	*er/trans/er* at 15	H	H	OH	OH at 10
106 Xylomaticin	*th/trans/th* at 15	H	OH	OH	OH at 10

Note: *er*=*erythro* and *th*=*threo*; db=double bond

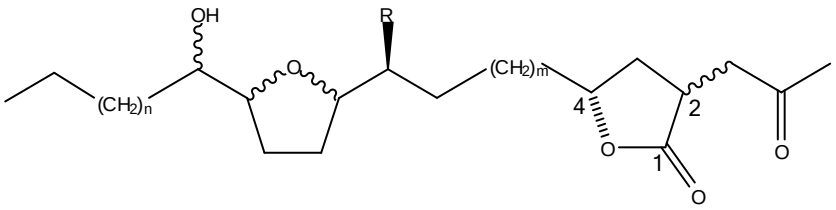
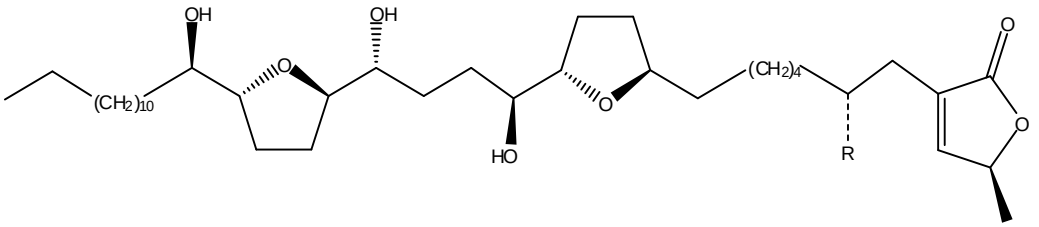


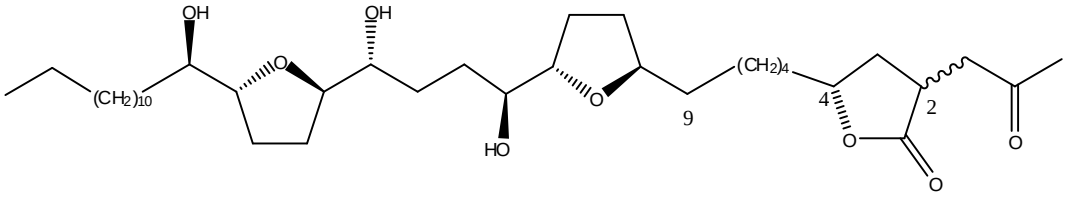
Table 2.5: Mono THF Acetogenins

Compound Name	**No. of Carbons**	**Position of Furan ring**	**R**	**Others and Position**
107 Annomontacin	37; m=11; n=12	At 17	OH	OH at 10; mixture of 2,4 *cis* and *trans*
108 Gigantetrocinone	35; m=3; n=16	At 9	H	OH at 17 & 18 (*th*) mixture of 2,4 *cis* and *trans*
109 Gonioneninone	37; m=7; n=14	At 13	OH	OH at 10; *cis* db at 21-22; mixture of 2,4 *cis* and *trans*
110 Isoannonacin	35; m=9; n=10	At 15	OH	OH at 10; mixture of 2,4 *cis* and *trans*
111 Xylomaticinone	37; m=9; n=12	At 15	OH	OH at 10; mixture of 2,4 *cis* and *trans*

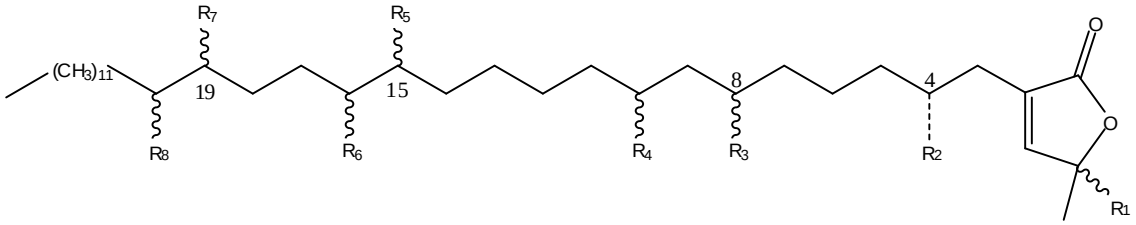
Note: *er*=*erythro* and *th*=*threo*; db=double bond



R=OH **112**
R=H **113**

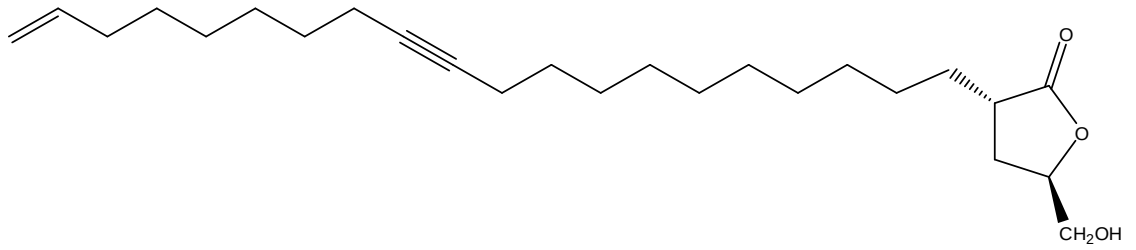


114 (mixture of *cis* and *trans*)



	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	Others and Position
115	H	H	H	OH	H	H	H	H	OH at 17&18 (*er*), cis db at 13-14
116	H	OH	OH	H	OH	OH	OH	OH	15&16 *er*, 19&20 *er*
117	H	OH	H	OH	H	H	H	H	OH at 17&18 (*th*)
118	OH	OAc	H	OH	OH	OH	OH	OH	15&16 *er*, 19&20 *er*

Note: *er*=*erythro* and *th*=*threo*; db=double bond



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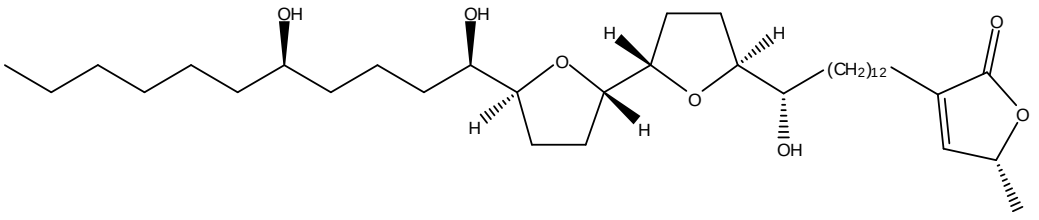
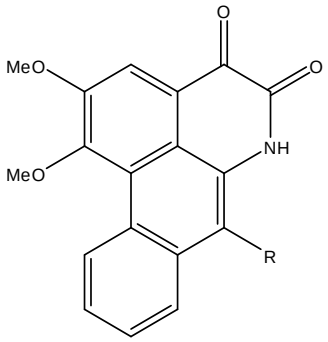
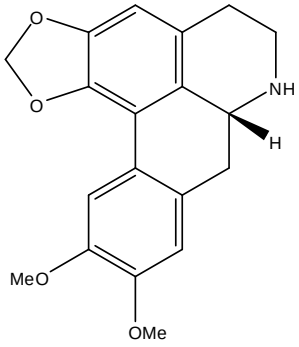


120

Alkaloids



R=Me 121
R=H 122



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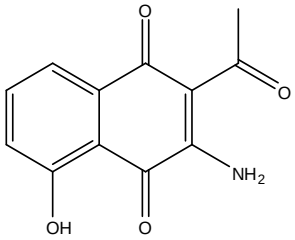

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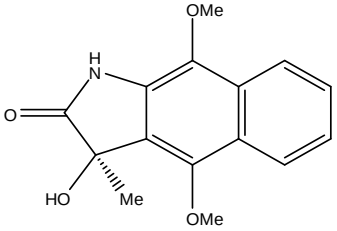

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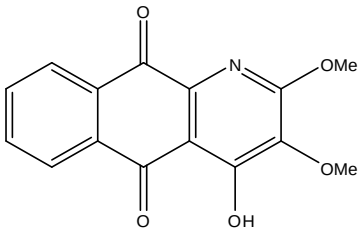

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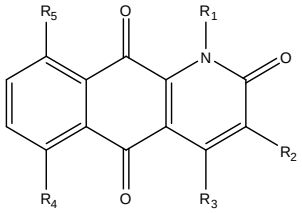
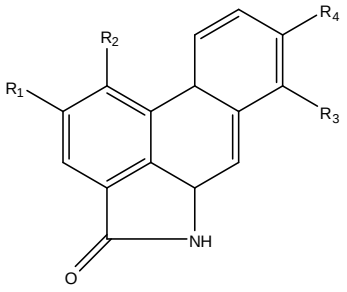
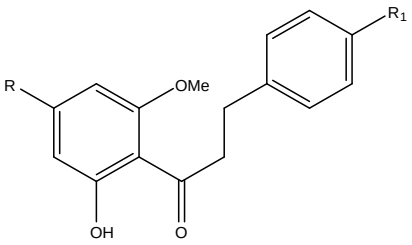


	R_1	R_2	R_3	R_4	R_5
127	H	H	Me	H	H
128	Me	OMe	Me	H	H
129	Me	OMe	EtOH	H	H
130	H	OMe	Me	OH	H
131	Me	OMe	Me	H	OH
132	H	OMe	Me	H	H

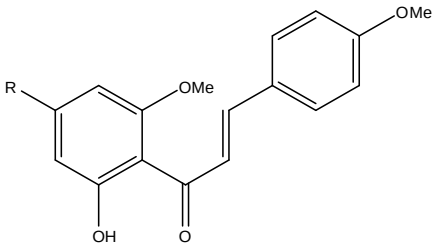


	R_1	R_2	R_3	R_4
133	OMe	OMe	OMe	H
134	OH	OMe	H	H
135	OMe	OH	OMe	H
136	OMe	OMe	H	OH

Chalcones

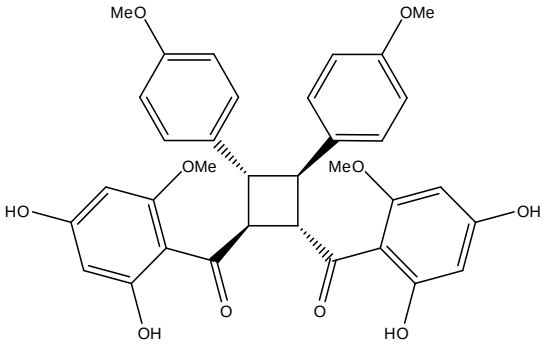


	R	R_1
137	OMe	OMe
138	OH	OMe
139	OH	OH



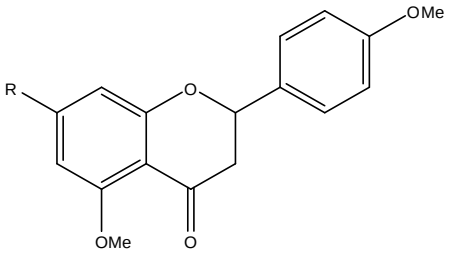
R=OMe **140**
R=OH **141**

Dihydrochalcones

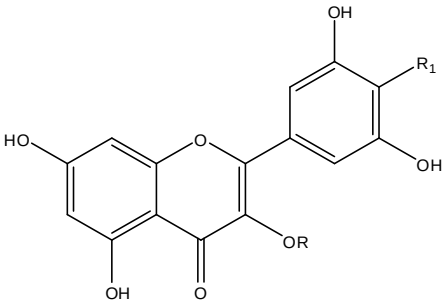


142

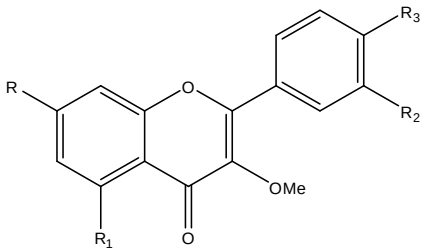
Flavonoids



R=OMe **143**
R=OH **144**

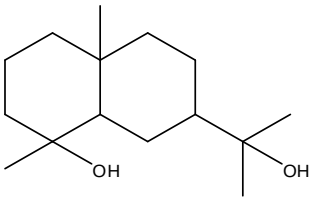


	R	R_1
145	rhamnose	OMe
146	Me	OH



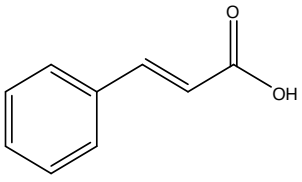
	R	R_1	R_2	R_3
147	OMe	OH	OMe	OMe
148	OMe	OMe	OMe	OMe
149	OH	OH	OH	OH
150	OMe	OH	OH	OH
151	OMe	OH	OMe	OH
152	OMe	OMe	OMe	OH
153	OMe	OMe	OH	OMe

Terpenes



154

Unclassified compounds



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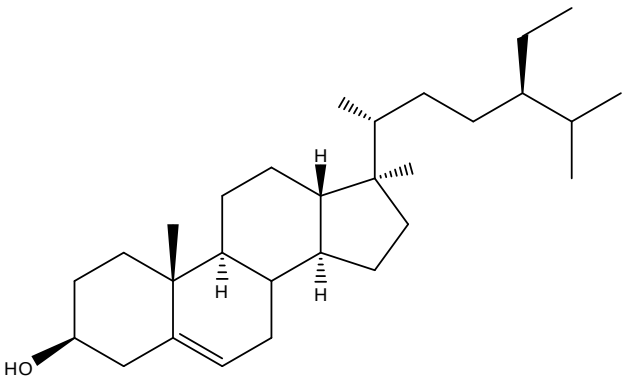

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2.7 Bioactivities

The phytochemical studies on *Goniothalamus* species have resulted in the isolation of two very distinct classes of lipophilic secondary metabolites, acetogenins and styryl-lactones. Both of them show cytotoxic against a broad array of human tumor cell lines including breast, colon, kidney and pancreatic carcinoma cells. They posses complex stereochemistry and exist in different stereoisomeric forms. The acetogenins and styryl-lactones have different biochemical pathways that take the molecular origin approaching or in the mitochondrial membrane and or mitochondrial respiratory system.[13]

A few alkaloids isolated from *G. marcanii* also exhibited significant cytotoxicity against several human tumor cell lines. From the phytochemical reports found so far on chemical constituents of *Goniothalamus* species tested for antitumor activity, *G. giganteus* is the one with most of cytotoxic acetogenins as shown in Table 2.6.

Table 2.6: Antitumor activity of *Goniothalamus* species

Species and Compound	**Cells**	**IC_{50}**
G. borneensis [41]		
Goniothalamin **1**	P388	0.75 μg/ml
	WEHI164	1.70 μg/ml
	MOLT-4	<1 μg/ml
G. donnaiensis [53,54]		
Donbutocin **24**	L1210	0.81 μg/ml
Donhexocin **23**	HCT-8	0.82 μg/ml
Goniodonin **85**	$HCT\text{-}8^{m}$	<10 μg/ml
G. gardneri [59]		
Gardnerilin A **116**	KB	>10 μg/ml
	HCT-8	>10 μg/ml
	Bel 7402	3.6 μg/ml
	KB	5.5 μg/ml

Species and Compound	**Cells**	**IC_{50}**
Gardnerilin B **117**	HCT-8	4.2 μg/ml
	Bel 7402	8.5 μg/ml
G. giganteus [27,28,69,70,75,77,78]		
4-Deoxyanomontacin **92**	A-549[a]	6.45×10^{-7} μg/ml
	MCF-7[b]	5.77×10^{-7} μg/ml
	HT-29[c]	1.41×10^{-1} μg/ml
	A-498[d]	1.50×10^{-1} μg/ml
	PC-3[e]	1.73×10^{-1} μg/ml
	PACA-2[f]	1.00×10^{-5} μg/ml
(*cis* and *trans*)-Annomontacinone **98**	HT-29	2.55×10^{-1} μg/ml
	PACA-2	6.78×10^{-1} μg/ml
cis-Gigantrionenin **102**	A-549	5.99×10^{-2} μg/ml
	MCF-7	2.68×10^{-1} μg/ml
	HT-29	6.94×10^{-6} μg/ml
	A-498	1.39×10^{-2} μg/ml
	PC-3	1.11×10^{-1} μg/ml
	PACA-2	1.15×10^{-1} μg/ml
4-Acetylgigantetrocin A **95**	A-549	$<10^{-2}$ μg/ml
	MCF-7	8.5×10^{-1} μg/ml
	HT-29	$< 10^{-2}$ μg/ml
	A-498	1.55×10^{-1} μg/ml
	PACA-2	$< 10^{-2}$ μg/ml
Annonacin **73**	PA1[g]	0.452 μg/ml
	SKOV3[h]	0.411 μg/ml
	HeLa[i]	0.219 μg/ml
	HeLa S3[j]	0.426 μg/ml
	MCF-7	0.433 μg/ml
	T-24[k]	0.324 μg/ml
	BCC-1[l]	0.427 μg/ml
Gigantransenin A **25**	A-549	0.16 μg/ml
Gigantransenin B **26**	A-549	0.21 μg/ml
	MCF-7	2.1×10^{-1} μg/ml
Gigantransenin C **27**	A-549	0.18 μg/ml
Goniotetracin **104**	A-549	3.9×10^{-1} μg/ml
	PC-3	2.1×10^{-1} μg/ml
	PACA-2	2.6×10^{-2} μg/ml

Species and Compound	**Cells**	**IC_{50}**
(2,4-cis and trans)-Gonioneninone **109**	PACA-2	4.5×10^{-2} μg/ml
Goniothalamicin **86**	A-549	2.80×10^{-1} μg/ml
Gonionenine **103**	PACA-2	4.5×10^{-2} μg/ml
Pyranicin **35**	A-549	2.8×10^{-1} μg/ml
	MCF-7	3.9×10^{-1} μg/ml
	A-498	1.8×10^{-1} μg/ml
	PC-3	4.1×10^{-1} μg/ml
	PACA-2	1.3×10^{-3} μg/ml
Pyragonicin **36**	PACA-2	5.8×10^{-2} μg/ml
Goniotrionin **87**	A-549	7.7×10^{-3} μg/ml
	MCF-7	5.3×10^{-6} μg/ml
	HT-29	3.4×10^{-1} μg/ml
	A-498	2.0×10^{-3} μg/ml
	PC-3	3.6×10^{-1} μg/ml
	PACA-2	5.4×10^{-3} μg/ml
G. griffithii [110]		
Goniothalamin **1**	HepG2	8.83 μM
	HepG2R	8 μM
Altholactone **5**	HepG2	0.7 μM
	HepG2R	6.17 μM
Goniodiol **2**	HepG2	10 μM
	HepG2R	8.33 μM
G. laoticus [88]		
3-Acetylaltholactone **64**	KB	2.9 μg/ml
	BC1	0.9 μg/ml
	NCI-H187 [o]	1.8 μg/ml
Goniotriol **60**	BC1	18.8 μg/ml
	NCI-H187	4.5 μg/ml
(+)-Altholactone **5**	KB	3.5 μg/ml
	BC1	0.8 μg/ml
	NCI-H187	0.6 μg/ml
(+)-Goniofufurone **11**	NCI-H187	9.5 μg/ml
	MCF-7	18.7 μg/ml
9-Deoxygoniopypyrone **15**	KB	22.7 μg/ml
	NCI-H187	2.6 μg/ml
	MCF-7	18.7 μg/ml

Species and Compound	**Cells**	**IC_{50}**
Howiinin A **52**	KB	16.6 μg/ml
	BC1	9.0 μg/ml
	NCI-H187	1.5 μg/ml
(–)-Nordicentrine **123**	KB	0.4 μg/ml
	NCI-H187	0.4 μg/ml
	MCF-7	2.9 μg/ml
G. marcanii [93]		
Marcanine A **127**	A-549	0.42 μM
	HT-29	0.42 μM
	MCF7	0.42 μM
	RPMI [p]	0.42 μM
	U251[q]	0.84 μM
Dielsiquinone **132**	A-549	0.11 μM
	HT-29	1.12 μM
	MCF7	0.11 μM
	RPMI	0.11 μM
	U251	0.37 μM
Marcanine B **128**	A-549	0.35 μM
	HT-29	2.12 μM
	MCF7	0.18 μM
	RPMI	0.70 μM
	U251	1.40 μM
Marcanine C **129**	A-549	1.00 μM
	HT-29	0.33 μM
	MCF7	1.00 μM
	RPMI	0.67 μM
Marcanine D **130**	A-549	0.04 μM
	HT-29	0.35 μM
	MCF7	0.08 μM
	RPMI	0.08 μM
	U251	0.28 μM
5-hydroxy-3-amino-2-aceto-1,4-naphthoquinone **124**	A-549	2.60 μM
	MCF7	2.60 μM
	RPMI	3.03 μM
	U251	3.03 μM

[a,o]human lung carcinoma; [b]human breast carcinoma; [c]human colon adenocarcinoma; [d]human kidney carcinoma; [e]human prostate adenocarcinoma; [f]human pancreatic carcinoma; [g,h]ovarian cancer cells; [i,j]cervical cancer; [k]bladder cancer; [l]skin cancer; [m]human colon adenocarcinoma; [n]hepatoma cell-line; [p]melanoma; [q]brain carcinoma.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Compounds of *Goniothalamus tapisoides*

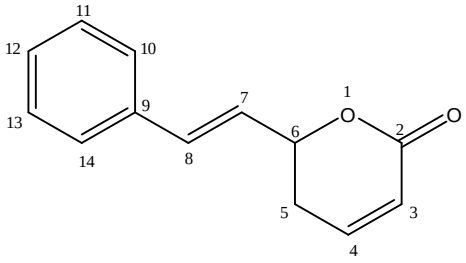
Eleven compounds have been isolated from the dichloromethane extracts of the stem bark of *Goniothalamus tapisoides* which were listed in Table 3.1. The isolation of pure compounds has been done through chromatographic methods (TLC and CC). The structures of the isolated compounds were then elucidated by nuclear magnetic resonance (NMR), mass spectrometry (MS), infrared spectroscopy (IR) and ultraviolet spectroscopy (UV).

Discussion on the structural elucidation of all the isolated compounds is briefly presented in the following section of this chapter.

Table 3.1: Isolated Compounds from *Goniothalamus tapisoides*

Compound name	Type of compound	Yield (mg)
Goniothalamin **1**	Styryl-lactones	1300
Goniomicin A **157**	-	12.3
Goniomicin B **158**	-	7.9
Goniomicin C **159**	Styryl-lactones	14.8
Goniomicin D **160**	Styryl-lactones	18.9
9-Deoxygoniopypyrone **15**	Styryl-lactones	4.4
Cinnamic acid **155**	-	4.0
Benzamide **161**	-	4.8
Liriodenine **40**	Alkaloids	1.2
Tapisoidin **162**	Alkaloids	3.2
Pterodondiol **164**	Terpenes	1100

3.1.1 Goniothalamin **1**



1

1 was isolated as white crystal needles (mp 80-82°C). [17] The mass spectrum showed a molecular ion peak at *m/z* 200, which corresponded to a molecular formula of $C_{13}H_{12}O_2$. The UV spectrum revealed maxima at 207, 255 and 284 nm. It showed strong bands in IR spectrum 1722, 1249, 752 cm^{-1} corresponding to the resonance of α, β-unsaturated δ-lactone moiety. [111]

The ^{1}H NMR spectrum showed a multiplet δ 7.16-7.26 referring to five aromatic protons (H-10 to H-14) of a *mono*-substituted phenyl ring. The two olefinic proton peaks at δ 6.60 (*d*, *J*=16.2 Hz) and δ 6.18 (*dd*, *J*=16.2 and 6.4 Hz) with a *trans* configuration belonged to H-8 and H-7 respectively. An allylic methylene signal observed as a multiplet at δ 2.32-2.37 (*m*) could be assigned to H-5 and a proton on a carbon bearing the oxygen of the lactone group appeared as a multiplet at δ 4.89-4.93 (*m*) belonged to H-6. The two proton of the allyl group resonating at δ 5.94 (*dd*, *J*=9.9, 1.4 Hz) and δ 6.75 (*ddd*, *J*=9.9, 4.6, 3.2 Hz) belonged to H-3 and H-4 respectively.

The ^{13}C NMR spectrum showed thirteen carbons; one methylene, ten methine and two quaternary carbon. The olefinic carbons C-7 and C-8 resonated at δ 125.9 and δ 133.0 respectively. A methylene carbon C-5 gave a peak at δ 29.8 meanwhile a methine carbon C-6 showed the peak at δ 78.1 due to the deshielding effect by the neighbouring oxygen atom. The signals for C-3 and C-4 resonated at δ 121.2 and δ 145.5 respectively.

Finally for aromatic carbon peak occured at δ 126.8 which attributed to the two aromatic carbons of C-10 and C-14, meanwhile the peak at δ 128.8 corresponding to the two aromatic carbons of C-11 and C-13. Another aromatic carbon peak appeared at δ 128.8 which could be assigned for C-12. The carbonyl carbon of the lactone appeared at δ 164.1.

Comparison of the spectral data with the literature values confirmed that **1** was indeed the styryl-lactone, goniothalamin.[17]

Table 3.2: ^{1}H, ^{13}C and HMBC Spectral Data of **1** in $CDCl_3$

Position	**δ_C (ppm)**	**δ_H (ppm), *J*(Hz)**	**HMBC (H→C)**
1			
2	164.1		
3	121.2	5.94 (1H, *dd*) *J*=9.9, 1.4	2, 5, 12
4	145.5	6.75 (1H, *ddd*) *J*=9.9, 4.6, 3.2	2, 5, 6, 8
5	29.8	2.32-2.37 (2H, *m*)	3, 4, 6, 7
6	78.1	4.89-4.93 (1H, *m*)	2, 4, 5, 7, 8
7	125.9	6.18 (1H, *dd*) *J*=16.2, 6.4	3, 5, 6, 9
8	133.0	6.6 (1H, *d*) *J*=16.2	6, 9, 10, 14
9	135.9		
10-14	126.8	7.16-7.26 (5H, *m*)	
	128.8		
	128.4		
	128.8		
	126.8		

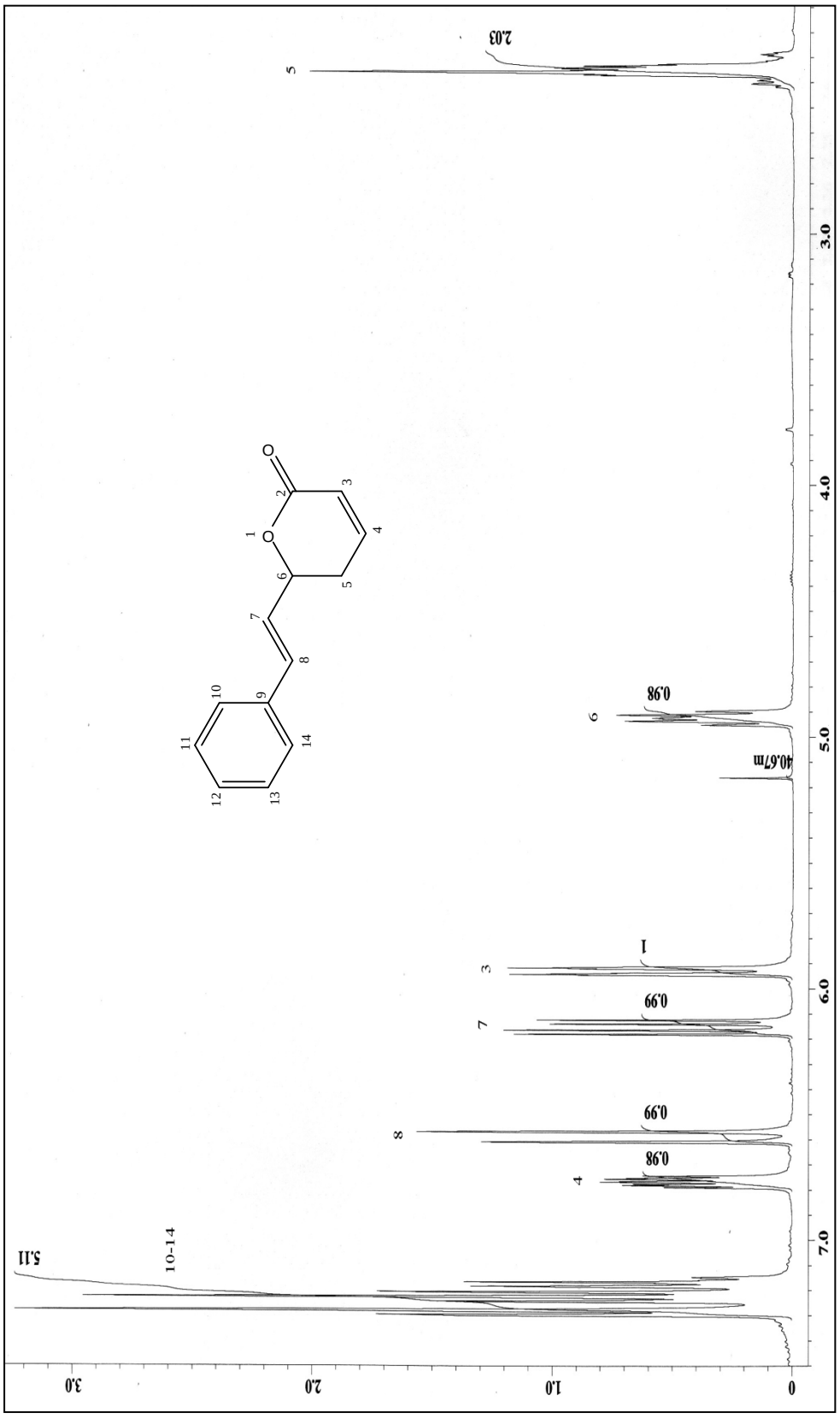


Figure 3.1: ^{1}H NMR Spectrum of **1**

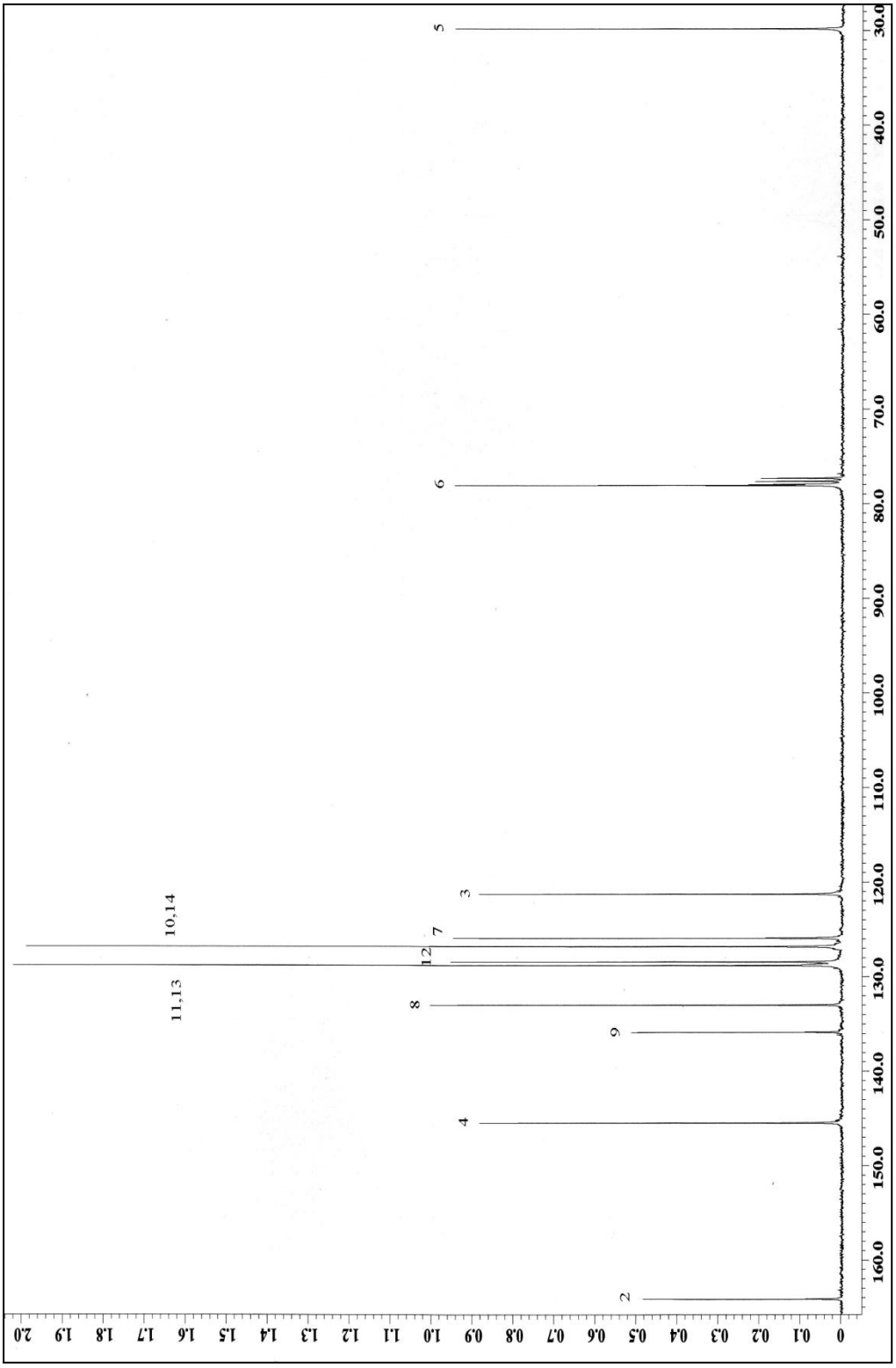
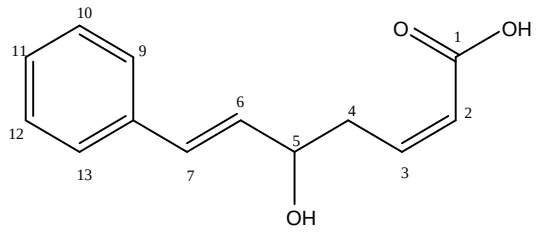


Figure 3.2: ^{13}C NMR Spectrum of **1**

3.1.2 Goniomicin A **157**



157

157 was isolated as yellowish amorphous solid. The mass spectrum showed a molecular ion peak at *m/z* 218, corresponding to a molecular formula of $C_{13}H_{14}O_3$. The IR spectrum showed strong absorptions bands of O-H stretching at 3344 cm^{-1}, C=O stretching at 1668 cm^{-1} and C-O stretching at 1329 cm^{-1}. [111] The UV spectrum revealed maximum at 206 and 251 nm.

The ^{1}H NMR spectrum showed the aromatic protons at δ 7.19-7.34 referring to five aromatic protons (H-9 to H-13) of a *mono*-substituted phenyl ring. Four olefinic protons peaks at δ 6.59, δ 6.20, δ 6.12 and δ 5.96 which belonged to H-7, H-6, H-3 and H-2 were observed. H-7 and H-6 were in *trans* configuration, while H-3 and H-2 were in *cis* configuration. The configurations were determined by a proton signal at δ 4.41 (*q*, *J*=6.6 Hz) was indicative of oxygen bearing methine proton H-5. Two allylic protons resonated at δ 2.76 (*m*) and δ 2.81 (*m*) belonged to H-4 and H-4'.

The ^{13}C and DEPT experiment further confirmed the presence of thirteen carbons; one methylene, ten methine and two quaternary carbon peaks appeared at δ 169.6 and δ 136.7 which were most probably belonged to C-1 and C-8 respectively. Four olefinic carbons; C-2, C-3, C-6 and C-7, resonated at δ125.6, δ 140.6, δ 131.9 and δ 129.9 respectively. C-3 resonated most downfield compared to the other olefinic carbons due to the α-β unsaturated resonance effect of carbonyl group at position C-1. The methylene carbon C-4 gave a peak at δ 36.61 meanwhile C-5 showed a peak at δ

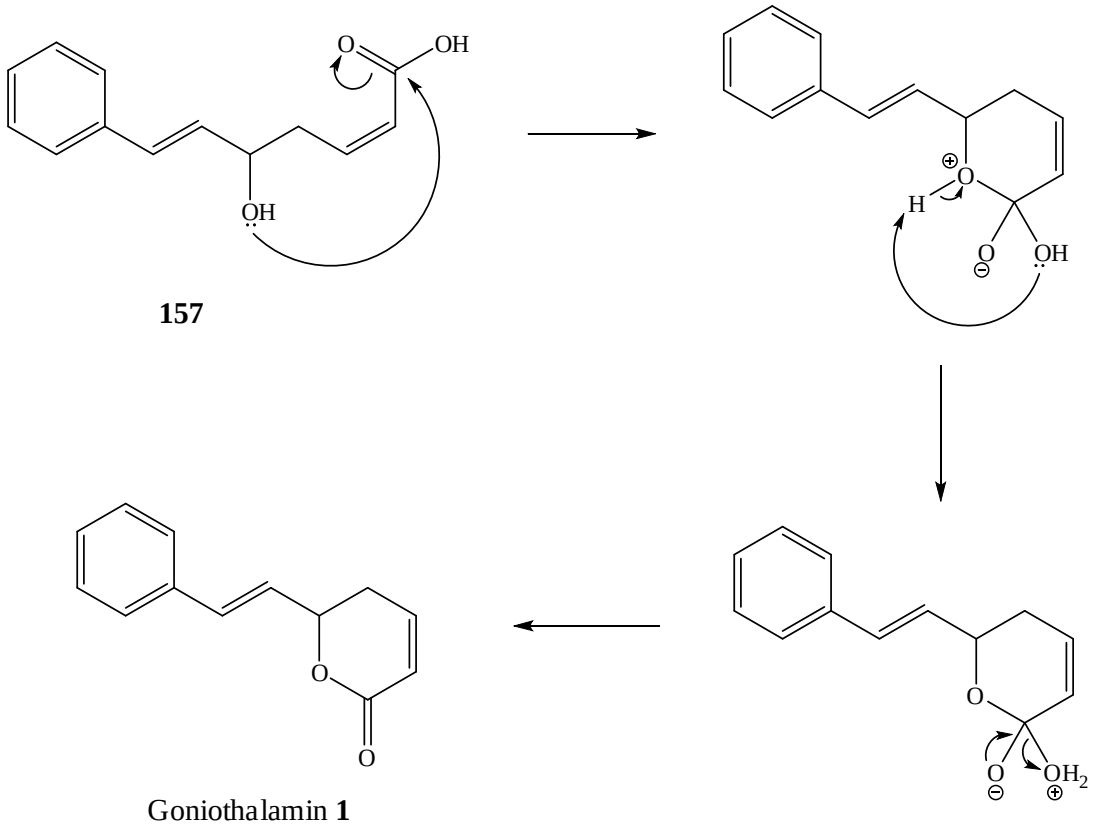
71.5 which were due to the deshielding effect by the neighbouring oxygen atom. Finally the five aromatic protons gave signals centred at δ 126.5-128.6 (C-9 to C-13).

The HMBC correlations of H-2, H-3 to C-1 suggested that the double bond was linked to C-1. The correlations of the two olefinic protons H-6, H-7 to C-5 and C-8 indicated the aromatic ring was connected to C-7.

157 is a new compound identified as (2*Z*,6*E*)-5-hydroxy-7-phenylhepta-2,6-dienoic acid, named as goniomicin A. **157** upon dehydration and cyclization form the styryl-lactone, goniothalamin **1** (compound A) (Scheme 3.1).

Table 3.3: ^{1}H, ^{13}C and HMBC Spectral Data of **157** in $CDCl_3$

Position	**δ_C (ppm)**	**δ_H (ppm), *J*(Hz)**	**HMBC (H→C)**
1	169.6		
2	125.3	5.96 (1H, *d*) *J*=11.9	1, 3, 4
3	140.6	6.12 (1H, *ddd*) *J*=11.9, 8.5, 3.5	1, 2
4	36.6	2.76 (1H, *m*)	3, 5, 6, 9
		2.81 (1H, *m*)	
5	71.5	4.41 (1H, *q*) *J*=6.6	7
6	131.9	6.20 (1H, *dd*) *J*=16.0, 6.6	4, 5, 8
7	129.9	6.59 (1H, *d*) *J*=16.0	5, 8, 9
1-OH		6.57 (OH, *br s*)	
5-OH		6.27 (OH, *br s*)	
8	136.7		
9-13	126.5	7.19-7.34 (5H, *m*)	
	128.6		
	127.6		
	128.6		
	126.5		



157

Goniothalamin **1**

Scheme 3.1: Dehydration and cyclization of **157** to form goniothalamin **1**

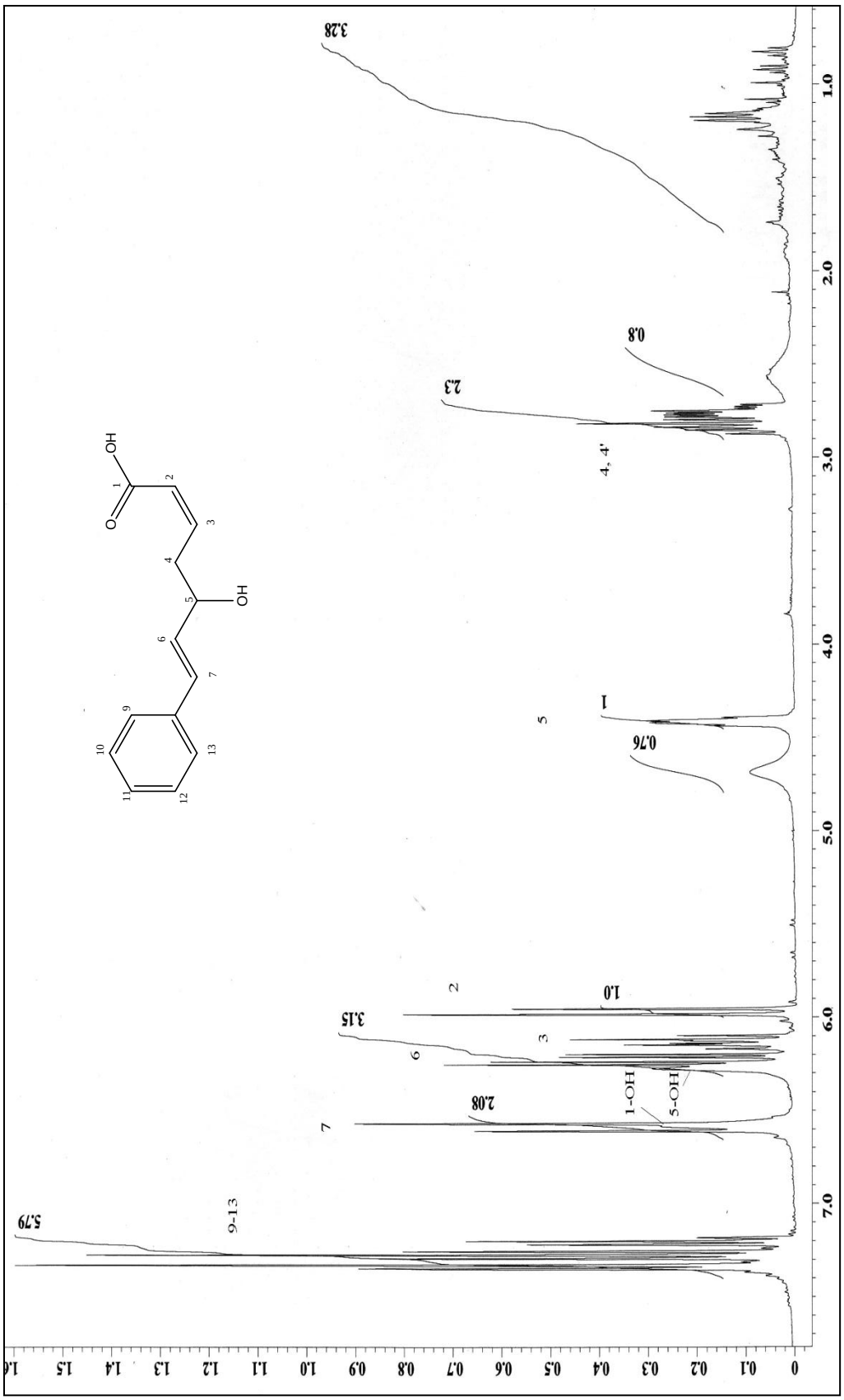


Figure 3.3: ^{1}H NMR Spectrum of **157**

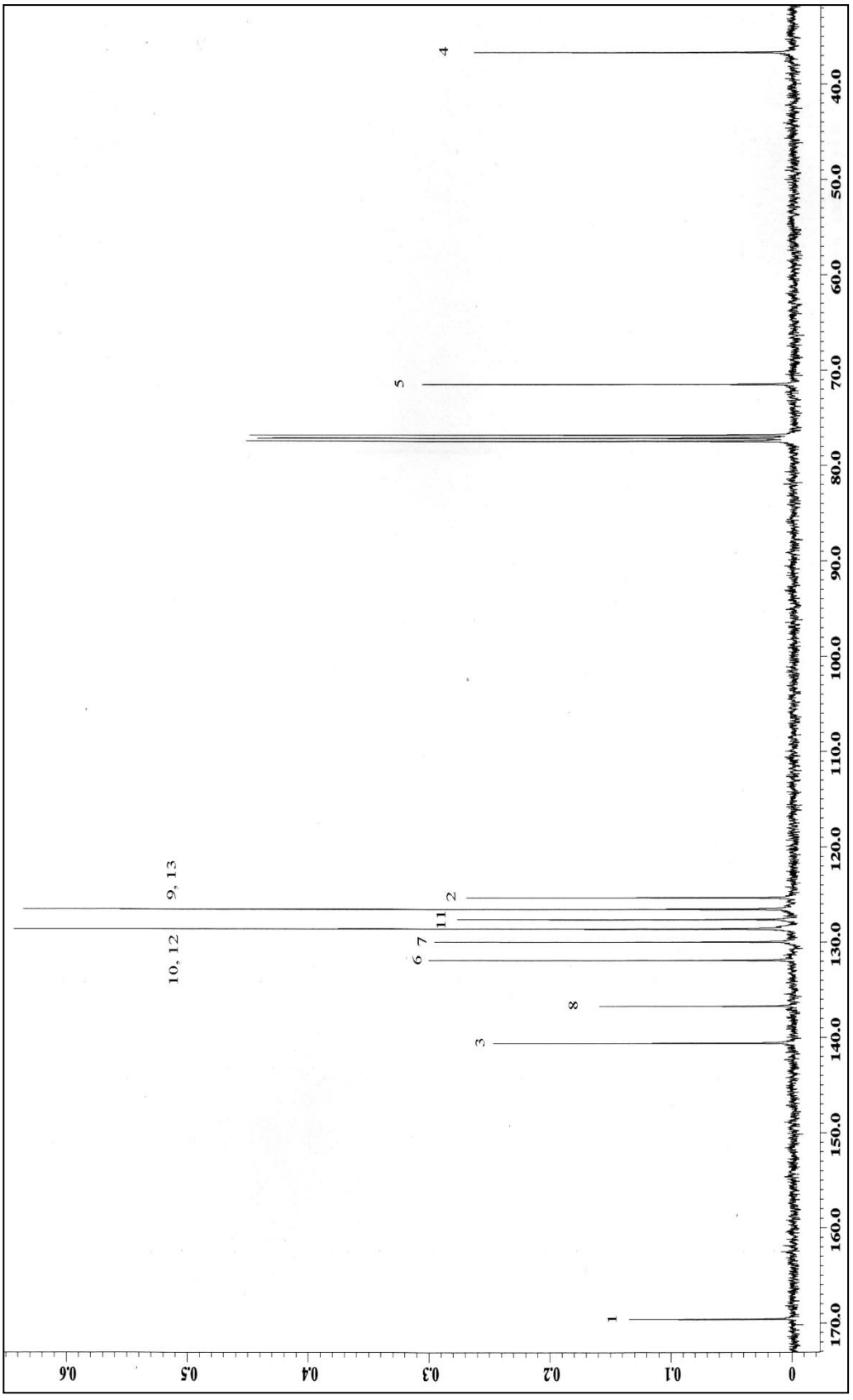


Figure 3.4: ^{13}C NMR Spectrum of **157**

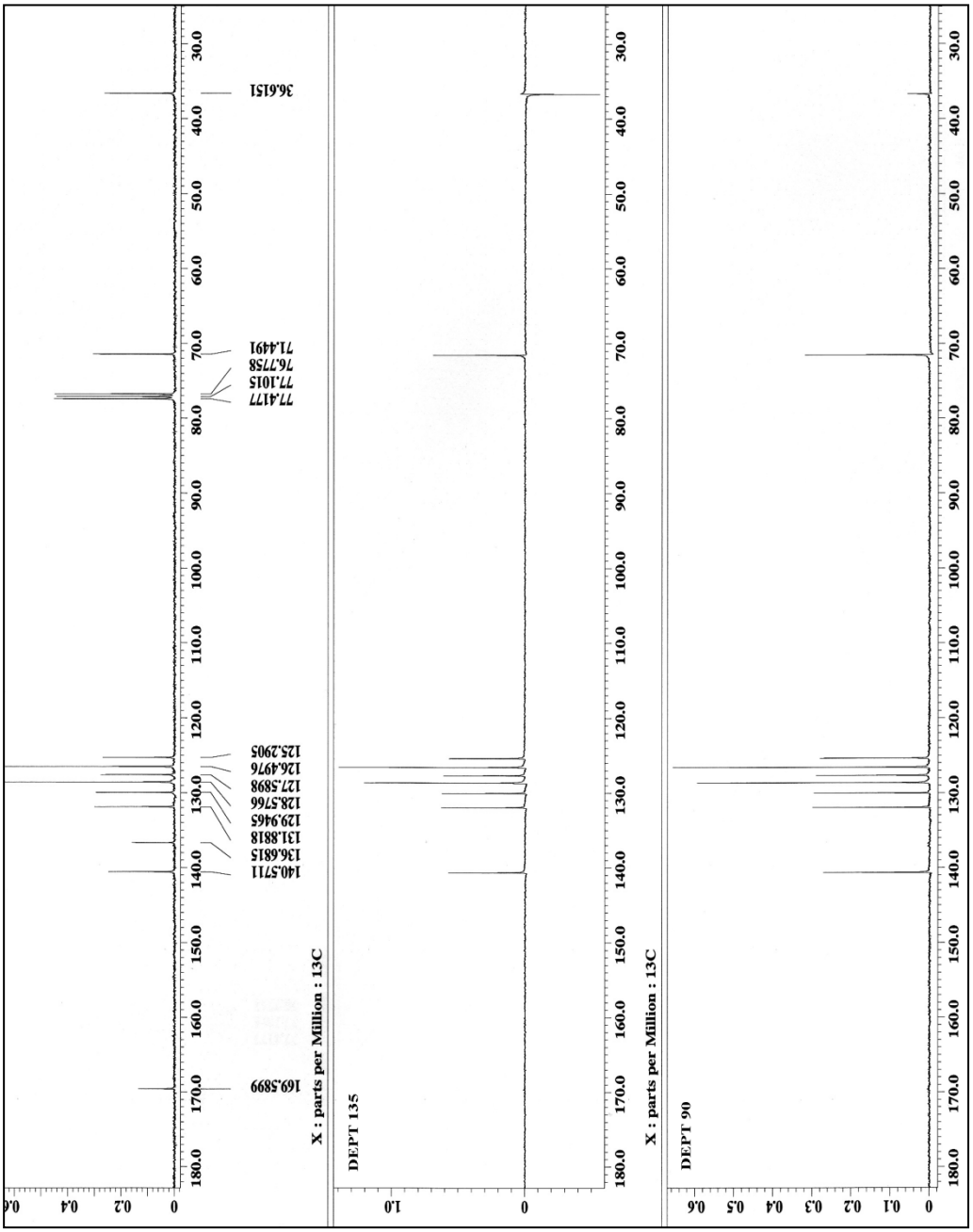


Figure 3.5: DEPT 135 and DEPT 90 Spectrum of **157**

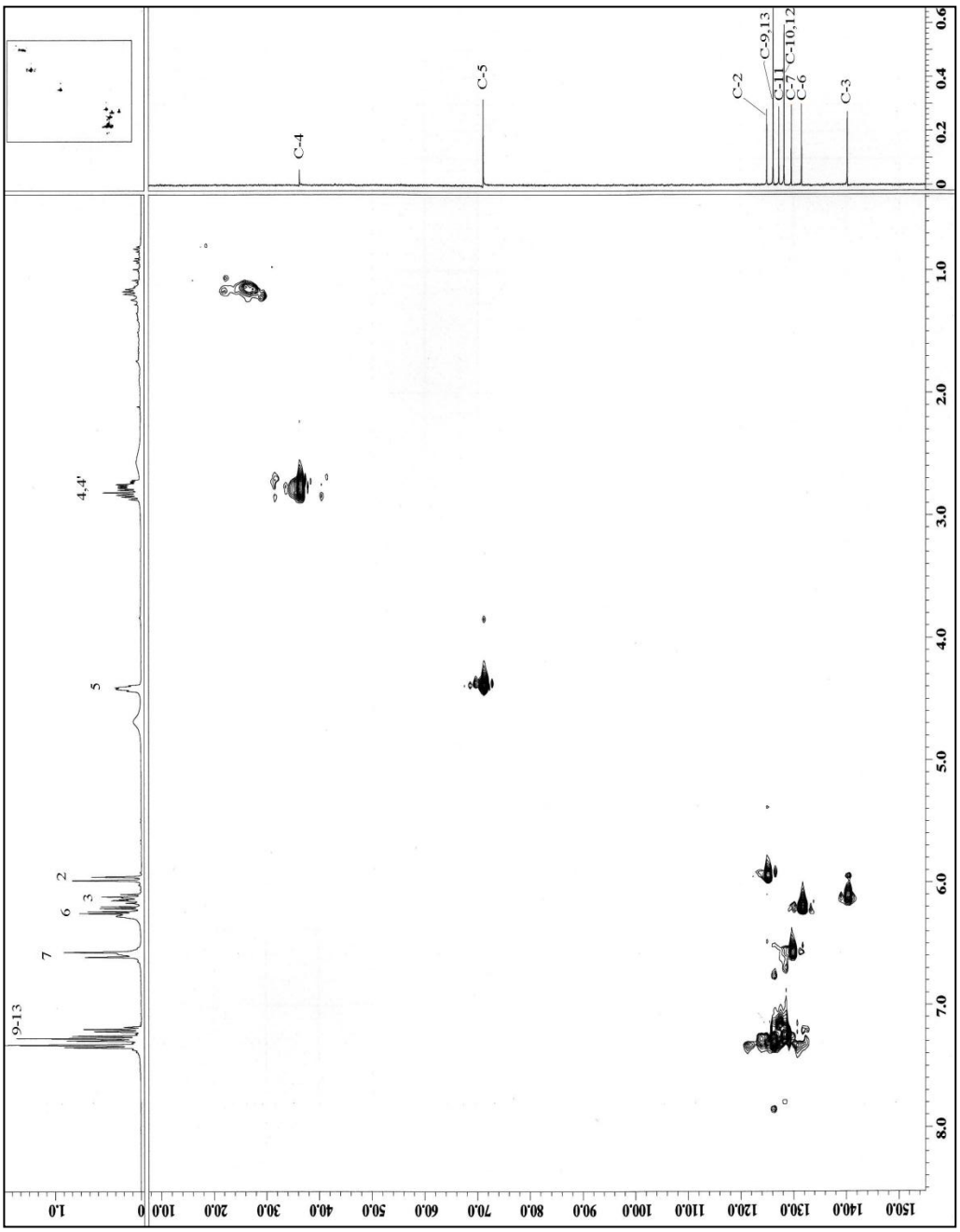


Figure 3.6: HSQC Spectrum of **157**

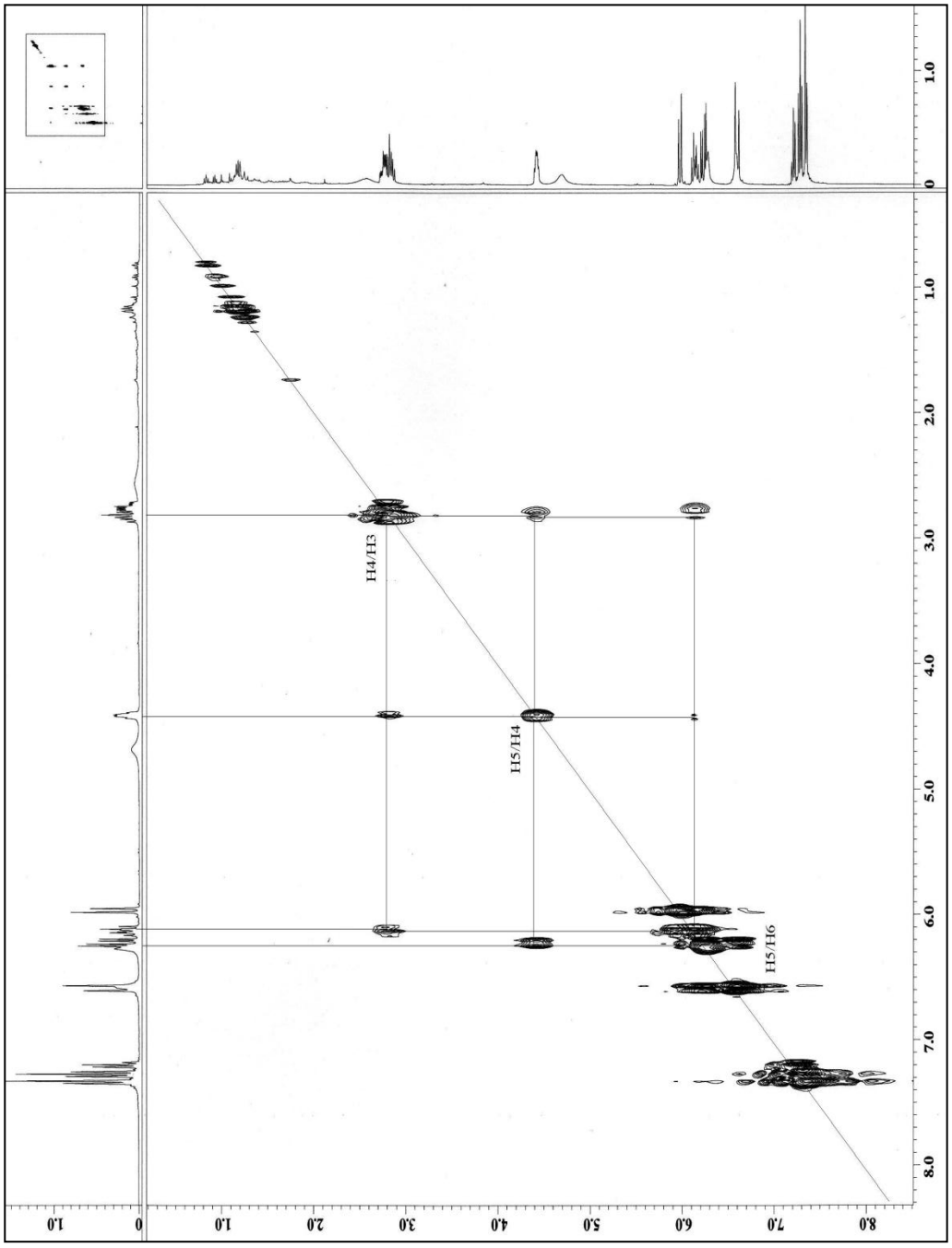


Figure 3.7: COSY Spectrum of **157**

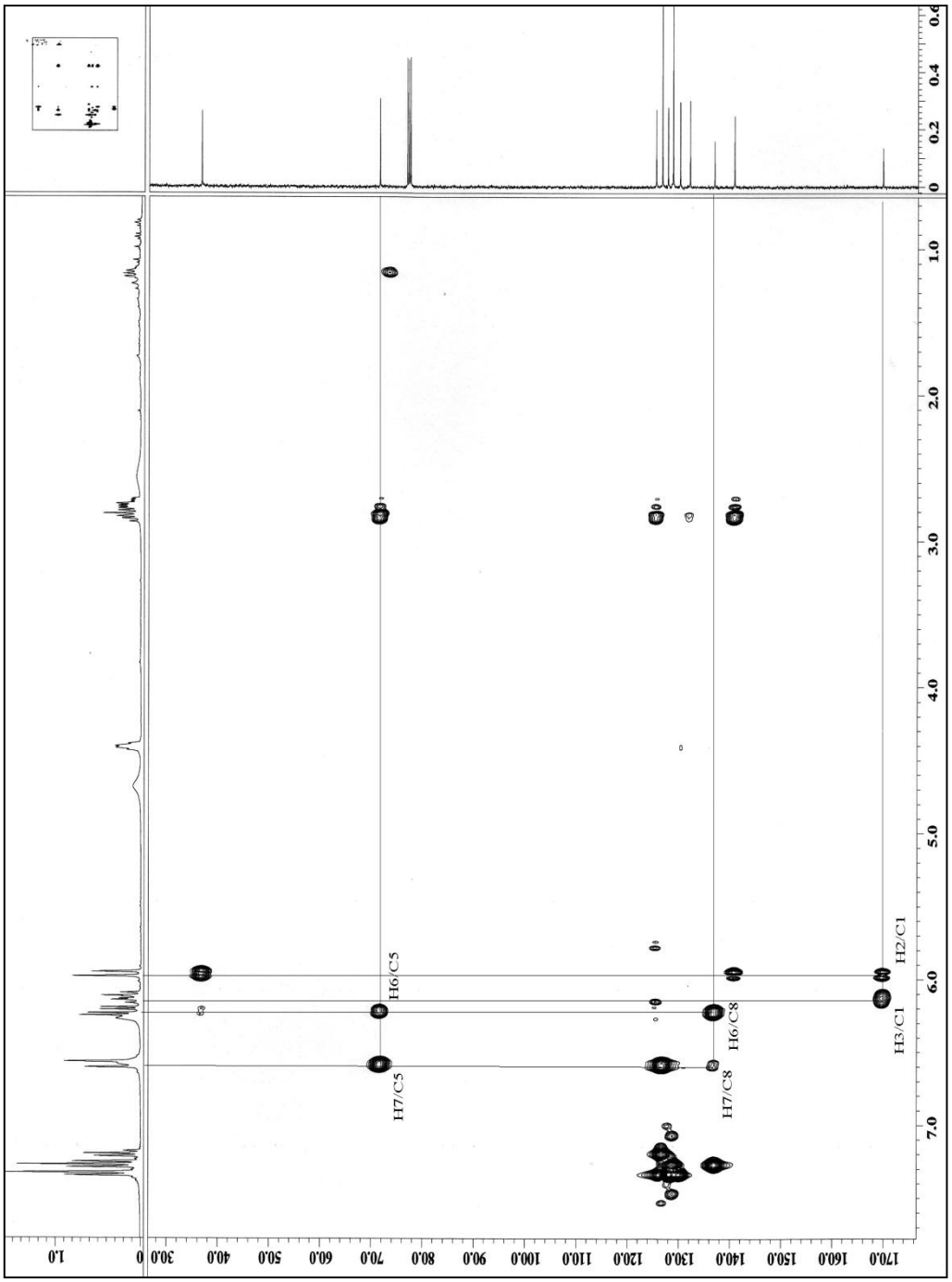
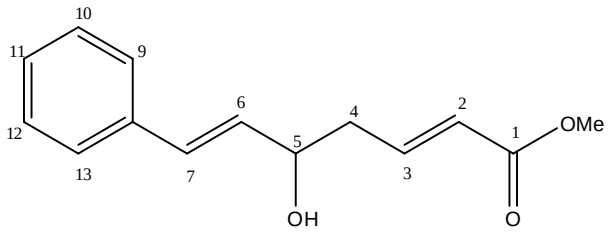


Figure 3.8: HMBC Spectrum of **157**

3.1.3 Goniomicin B **158**



158

158 was isolated as yellowish amorphous solid. The mass spectrum showed a molecular ion peak at *m/z* 232, corresponding to a molecular formula of $C_{14}H_{16}O_3$. **158** is similar to **157**, except for the methoxyl group at C-1. The IR spectrum showed a strong conjugated carbonyl stretch of an ester at 1718 cm^{-1}. [111] The UV spectrum showed absorption bands at 207 and 251 nm suggesting the presence of an aromatic ring. [111]

The ^{1}H NMR spectrum showed a distinct methoxyl group at δ 3.72 (*s*) which is most probably positioned at C-1. H-3 resonated at δ 7.00 (*dt*, *J*=15.1, 7.3) was more deshielded in **158** compared to **157** due to methoxyl group attachment. Coupling constant of H-7, H-6 and H-3, H-2 is 15-16 Hz, this showed that both of it having *trans* configuration.

The ^{13}C NMR spectrum of this compound is very similar with **157**. The chemical shift of C-1 (δ 166.8) which is more shielded as compared to C-1 of **157** (δ 169.6). This is because C-1 is attached to a methoxyl while C-1 of **157** is attached to a hydroxyl group. Another difference were the chemical shifts of C-3 and C-4 is more deshielded by 5 ppm as (δ 144.7, δ 40.2) respectively compared to those of **157** (δ 140.6, δ 36.6).

The HMBC correlations between methoxyl group and C-1 showed that the methoxyl group was linked to C-1. The HMBC cross peaks of **157** and **158** were also

similar, thus suggesting that the structure had the same chain but the functional group attached to C-1 was different.

Therefore, **158** is a new compound identified as (2*E*, 6*E*)-methyl 5-hydroxy-7-phenyl-2, 6-heptadienoate, named as goniomicin B.

Table 3.4: ^{1}H, ^{13}C and HMBC Spectral Data of **158** in $CDCl_3$

Position	**δ_C (ppm)**	**δ_H (ppm), *J*(Hz)**	**HMBC (H→C)**
1	166.8		
2	123.9	5.94 (1H, *d*) *J*=15.1	1, 4
3	144.7	7.00 (1H, *dt*) *J*=15.1, 7.3	1, 4
4	40.2	2.54 (2H, *dt*) *J*=6.4, 1.4	2, 3, 5, 6
5	71.6	4.44 (1H, *q*) *J*=6.4	3, 7
6	130.9	6.22(1H, *dd*) *J*=16, 6.4	5, 8
7	131.3	6.61 (1H, *d*) *J*=16	5, 9, 11
8	136.3		
9-13	128.0	7.24-7.38 (5H, *m*)	
	128.6		
	126.5		
	128.6		
	126.5		
1-OMe	51.6	3.72 (3H, *s*)	1

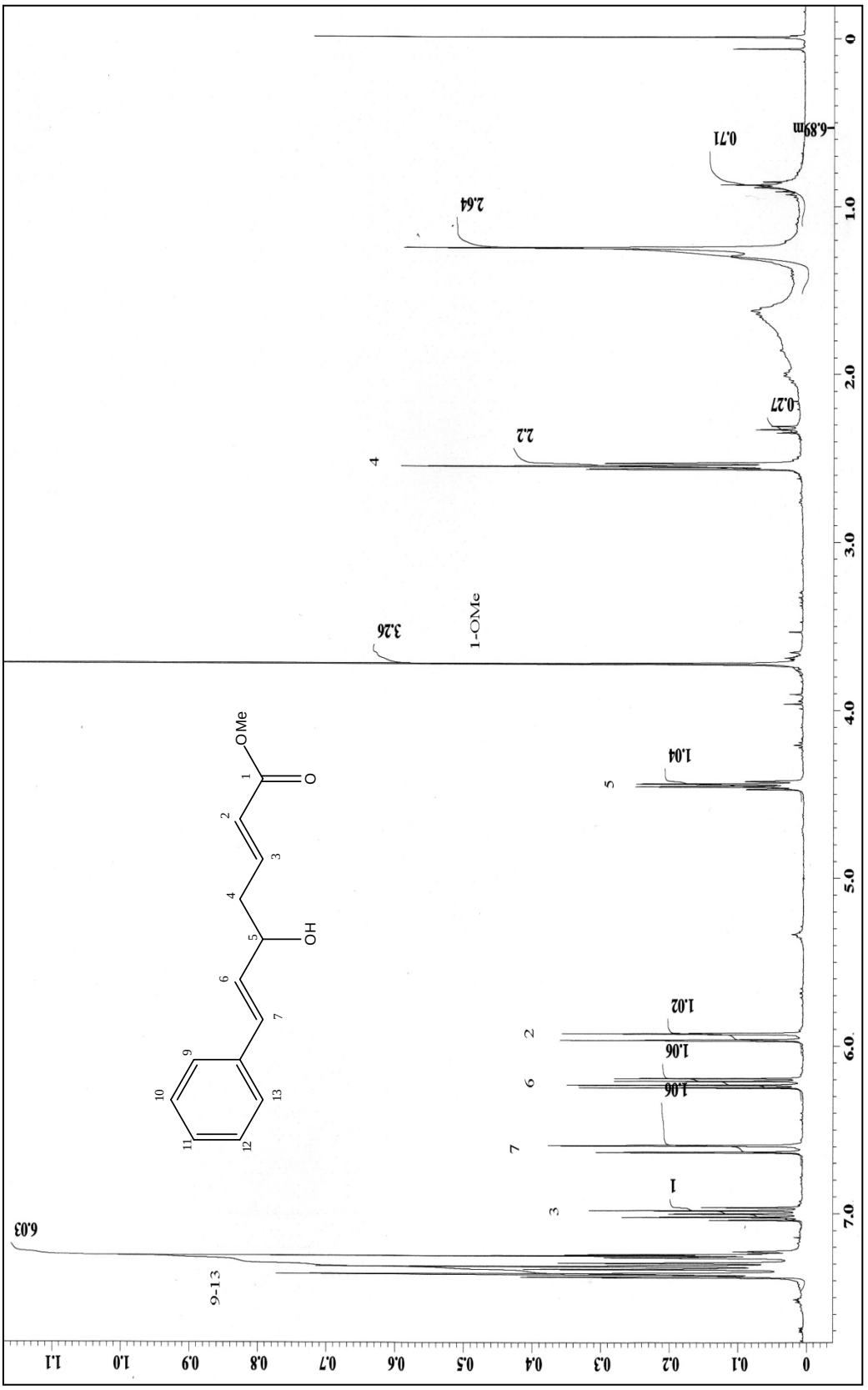


Figure 3.9: ^{1}H NMR Spectrum of **158**

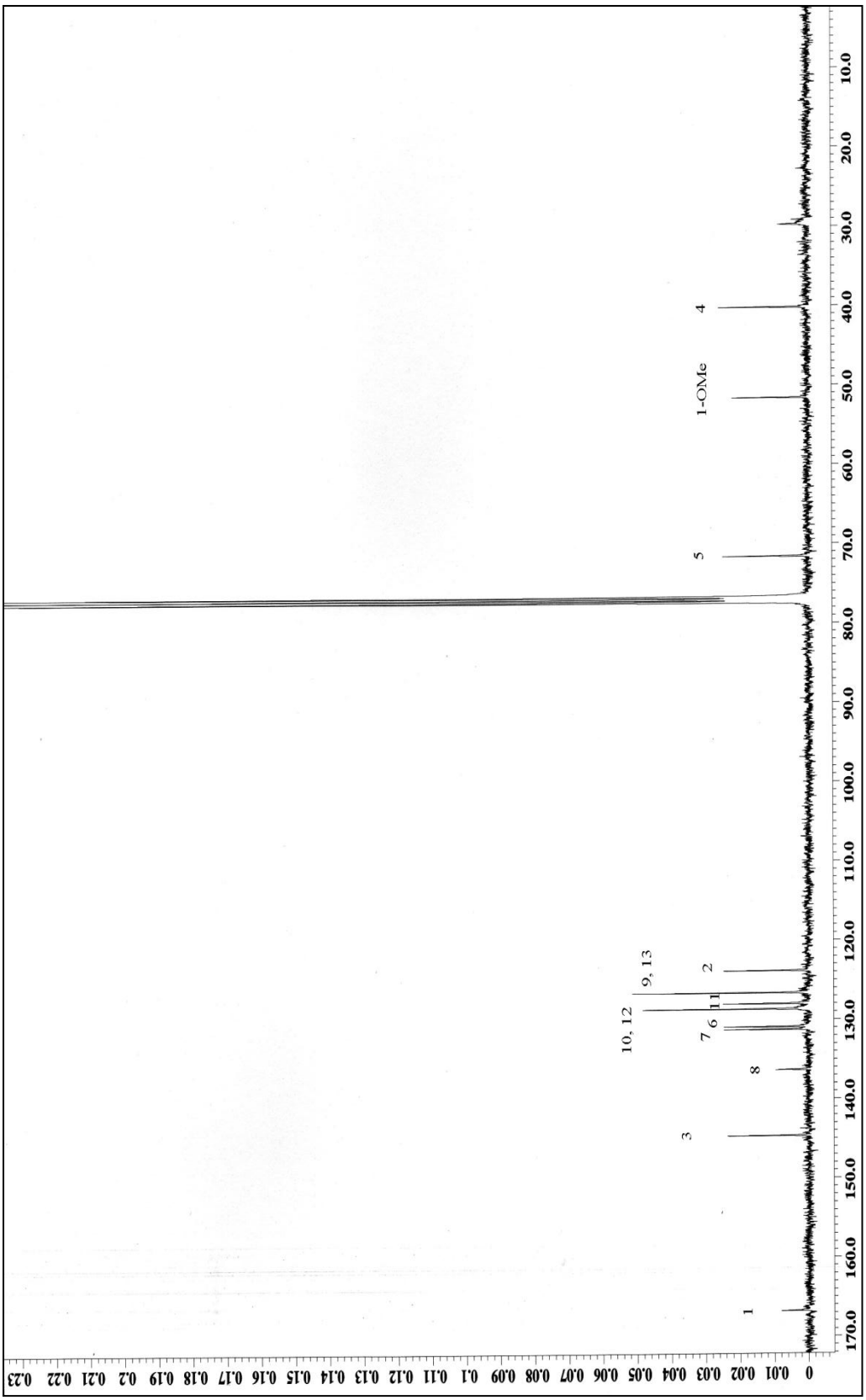


Figure 3.10: ^{13}C NMR Spectrum of **158**

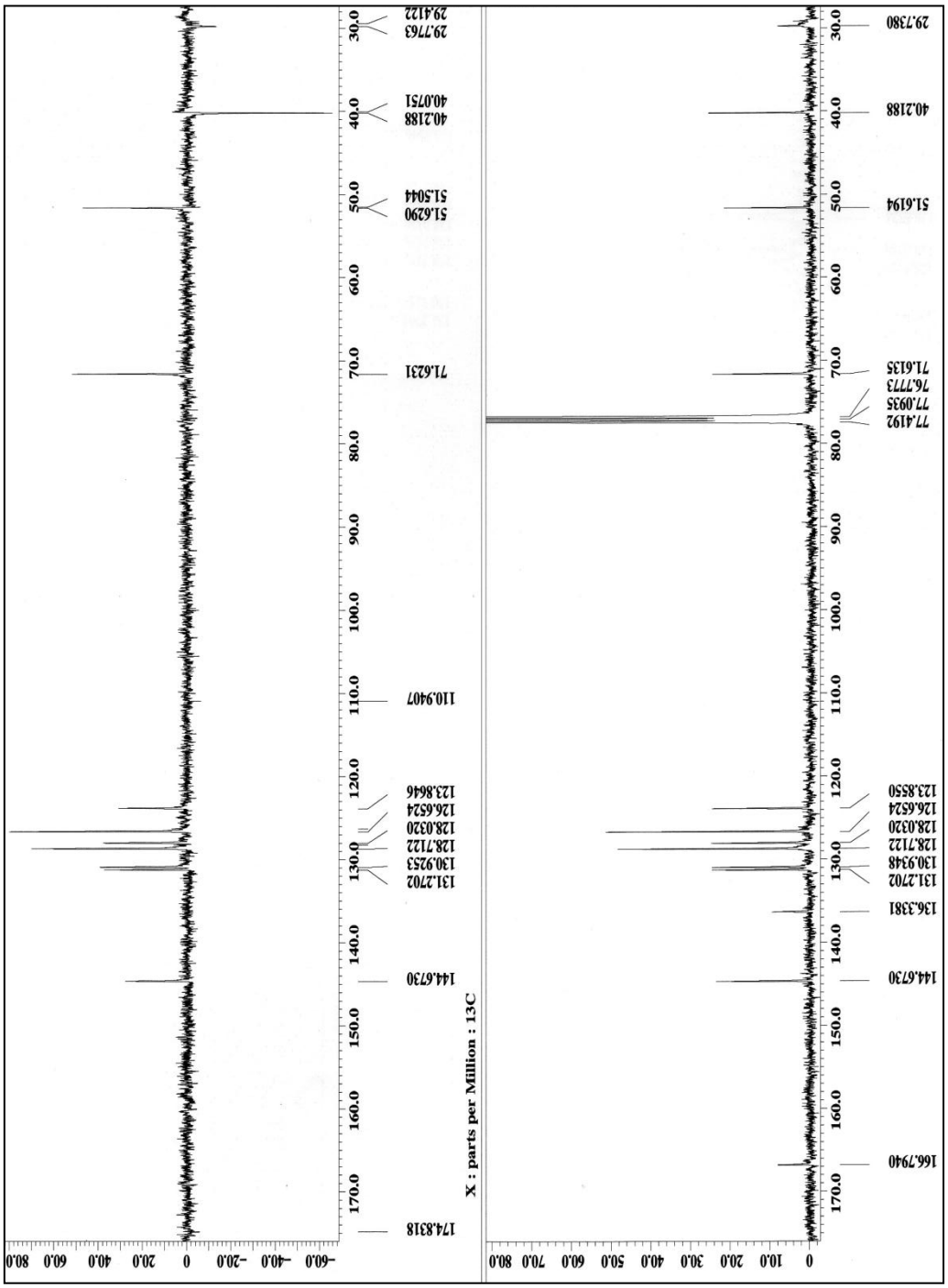


Figure 3.11: DEPT 135 Spectrum of **158**

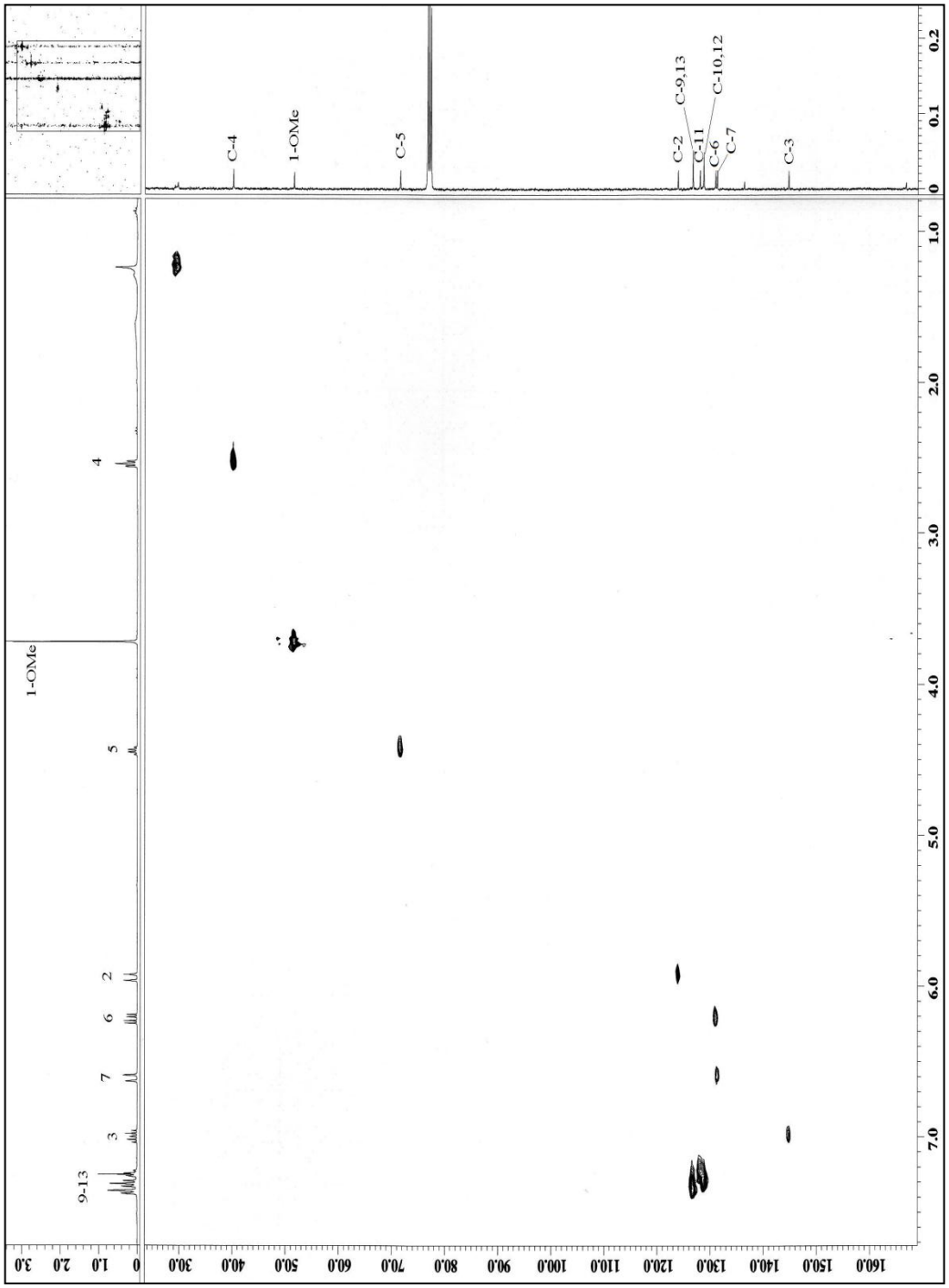


Figure 3.12: HSQC Spectrum of **158**

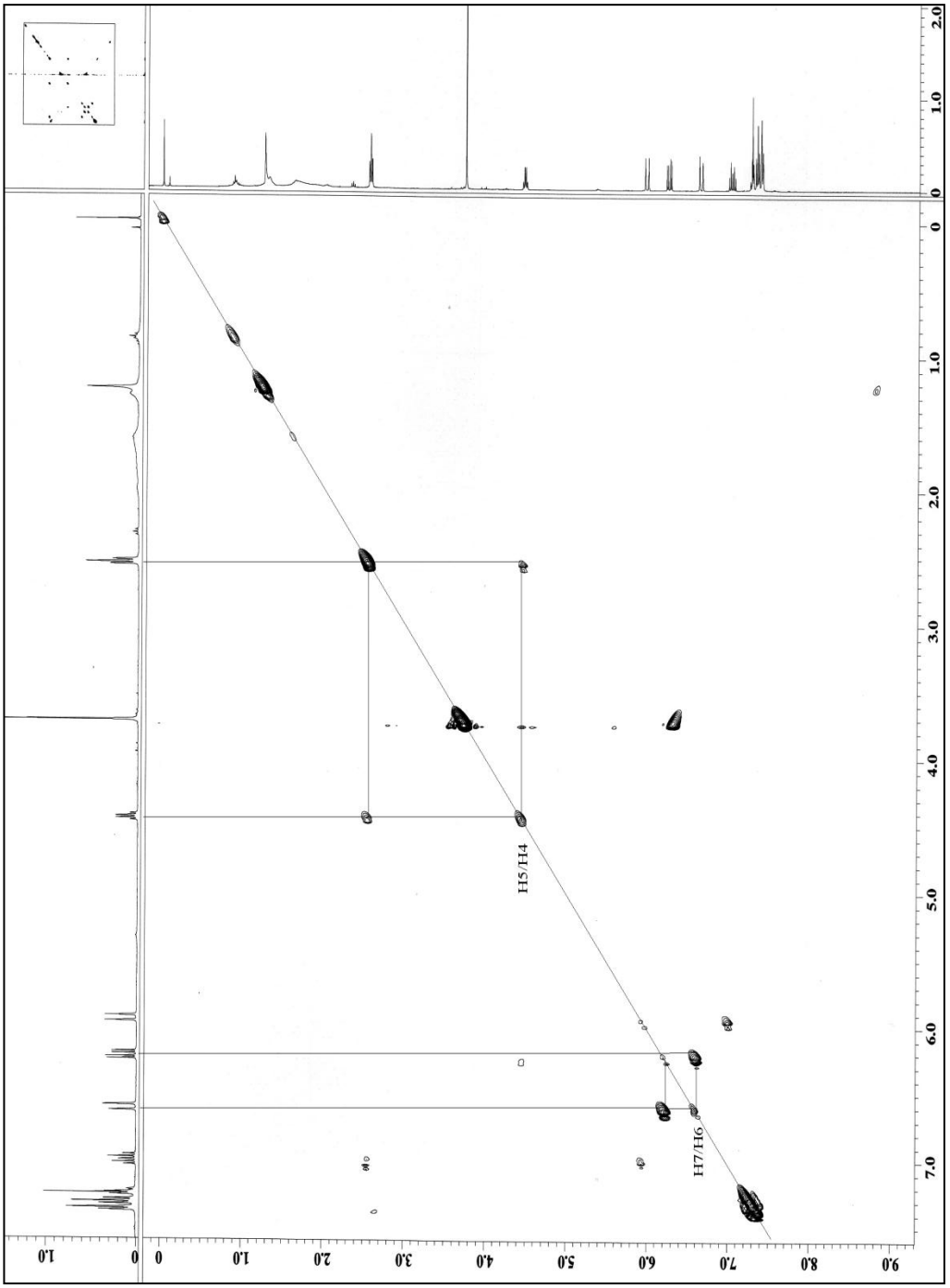


Figure 3.13: COSY Spectrum of **158**

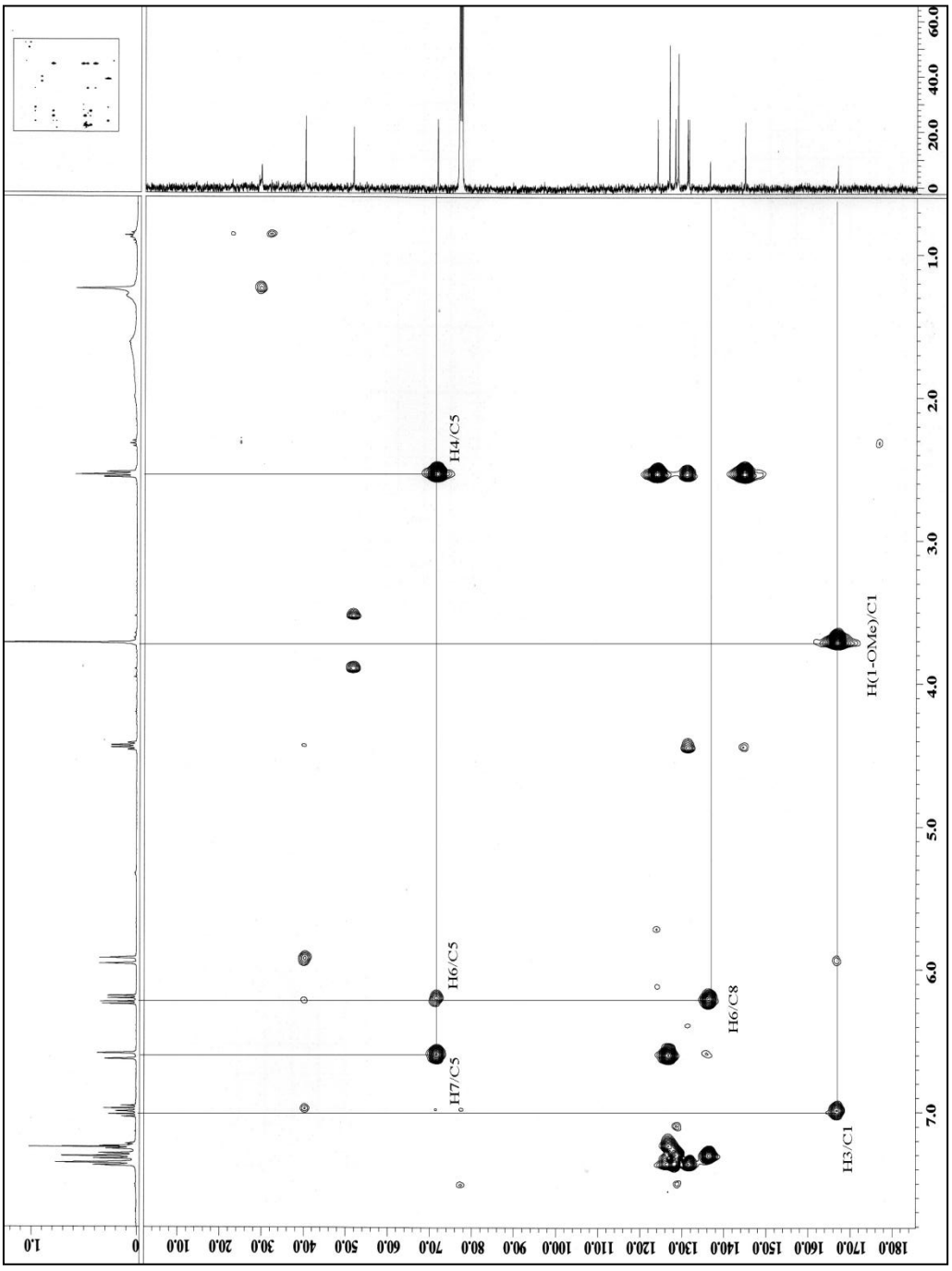
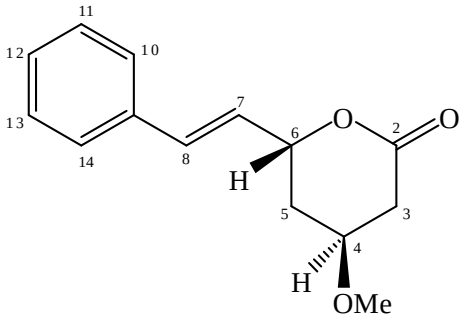


Figure 3.14: HMBC Spectrum of **158**

3.1.4 Goniomicin C **159**



159

159 was isolated as yellowish amorphous solid. The mass spectrum showed a molecular ion peak at *m/z* 232, corresponding to a molecular formula of $C_{14}H_{16}O_3$. In the UV region, it absorbed at 206 and 251 nm. Its IR spectrum showed strong absorptions bands of C-H stretching at 2925 cm^{-1}, C=O stretching at 1731 cm^{-1} and C-O stretching at 1241 and 1090 cm^{-1}. [111]

The ^{1}H NMR spectrum showed the aromatic proton signals centred at δ 7.24-7.37 belonging to five aromatic protons (H-10 to H-14) of a *mono*-substituted phenyl ring. Two olefinic protons peaks at δ 6.68 (*d*, *J*=16.0) and δ 6.18 (*dd*, *J*=16.0, 6.4) were attributable to H-8 and H-7 respectively. A distinct methoxyl group showed signal at δ 3.36 (*s*) which was most probably positioned at H-4. H-5 and H-5' were non-equivalent methylene protons resonated at δ 1.87 (*dt*, *J*=11.4, 3.2) and δ 2.18 (*dt*, *J*=14.6, 3.2). An allylic methylene was observed at δ 2.73 (*m*) could be assigned to the H-3. A proton on a carbon bearing oxygen of the lactone group appeared as a multiplet at δ 5.20 (*m*) belonged to the H-6.

The ^{13}C NMR spectrum showed fourteen carbons; one methyl, two methylene, nine methine and two quaternary carbon. Two olefinic carbons; C-7 and C-8 resonated at δ 126.6 and δ 132.5 respectively. Meanwhile C-4 and C-6 showed downfield peaks at δ 71.4 and δ 76.2 respectively due to the deshielding effect by the neighbouring oxygen

atom. Finally four aromatic carbon peaks were observed at δ 126.5-128.6 attributed to the five aromatic carbons of C-10 to C-14.

H-3/H-4, H-4/H-5, H-5/H-6, H-6/H-7 and H-7/H-8 cross peaks were observed in COSY spectrum therefore suggesting the sequence of H-3 to H-7. The HMBC correlations of the two olefinic protons H-7, H-8 to C-6, C-9 and C-10 indicating that the aromatic ring was connected to C-9.

Therefore, **159** is a new compound identified as the styryl-lactone (*E*)-4-methoxy-6-styryltetrahydro-2*H*-pyran-2-one, named as goniomicin C.

Table 3.5: ^{1}H, ^{13}C and HMBC Spectral Data of **159** in $CDCl_3$

Position	**δ_C (ppm)**	**δ_H (ppm), *J*(Hz)**	**HMBC (H→C)**
1			
2	169.7		
3	35.7	2.73 (2H, *dt*) *J*=4.4, 1.4	
4	71.4	3.82 (1H, *quin*) *J*=4.4	
5	33.7	1.87 (1H, *dt*) *J*=11.4, 3.2	
		2.18 (1H, *dt*) *J*=14.6, 3.2	
6	76.2	5.20 (1H, *m*)	
7	126.6	6.18 (1H, *dd*) *J*=16.0, 6.4	6, 9
8	132.5	6.68 (1H, *d*) *J*=16.0	6, 10, 14
9	136.0		
10-14	126.7	7.24-7.37 (5H, *m*)	
	128.8		
	128.3		
	128.8		
	126.7		
4-OMe	56.3	3.36 (3H, *s*)	4

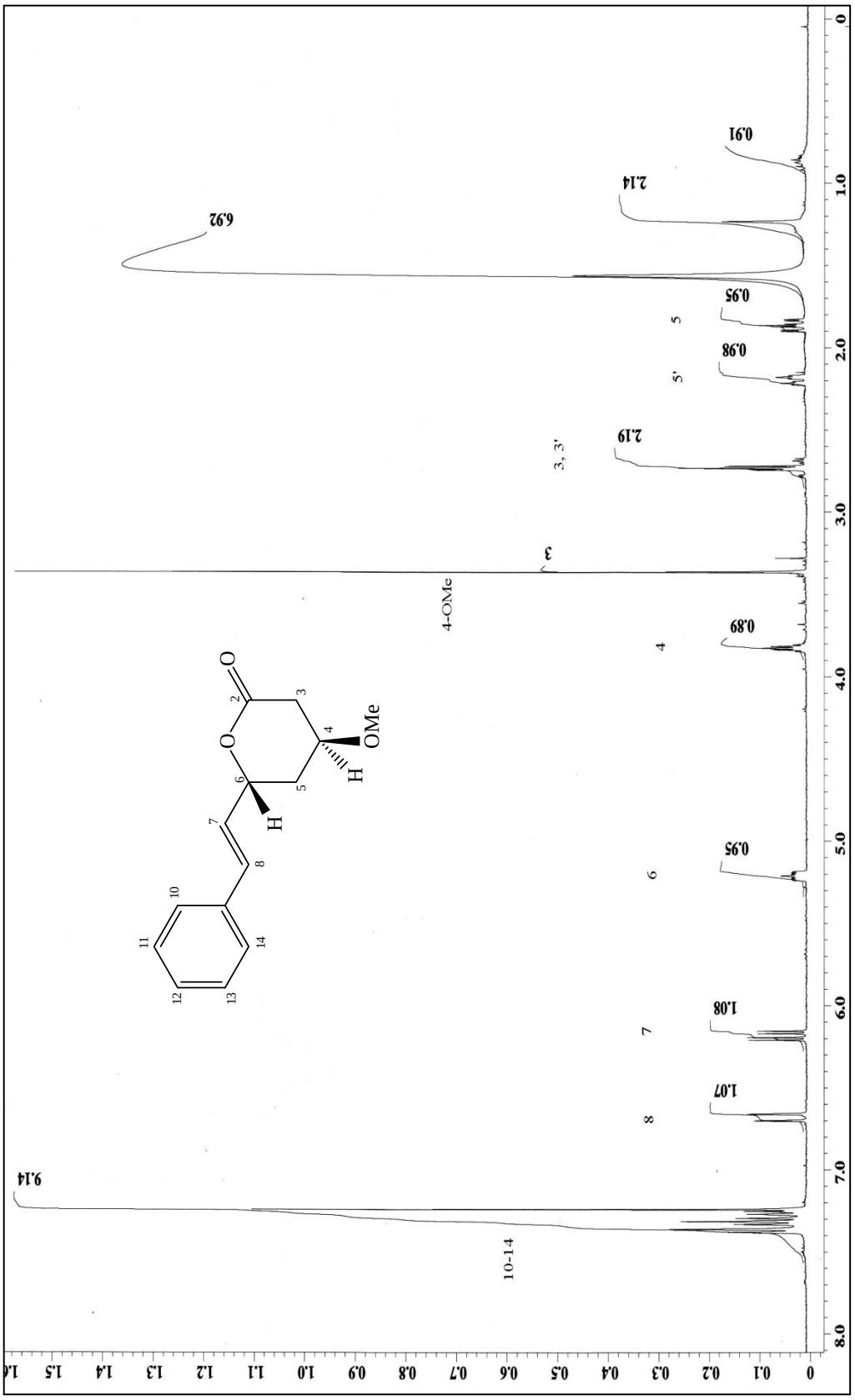


Figure 3.15: ^{1}H NMR Spectrum of **159**

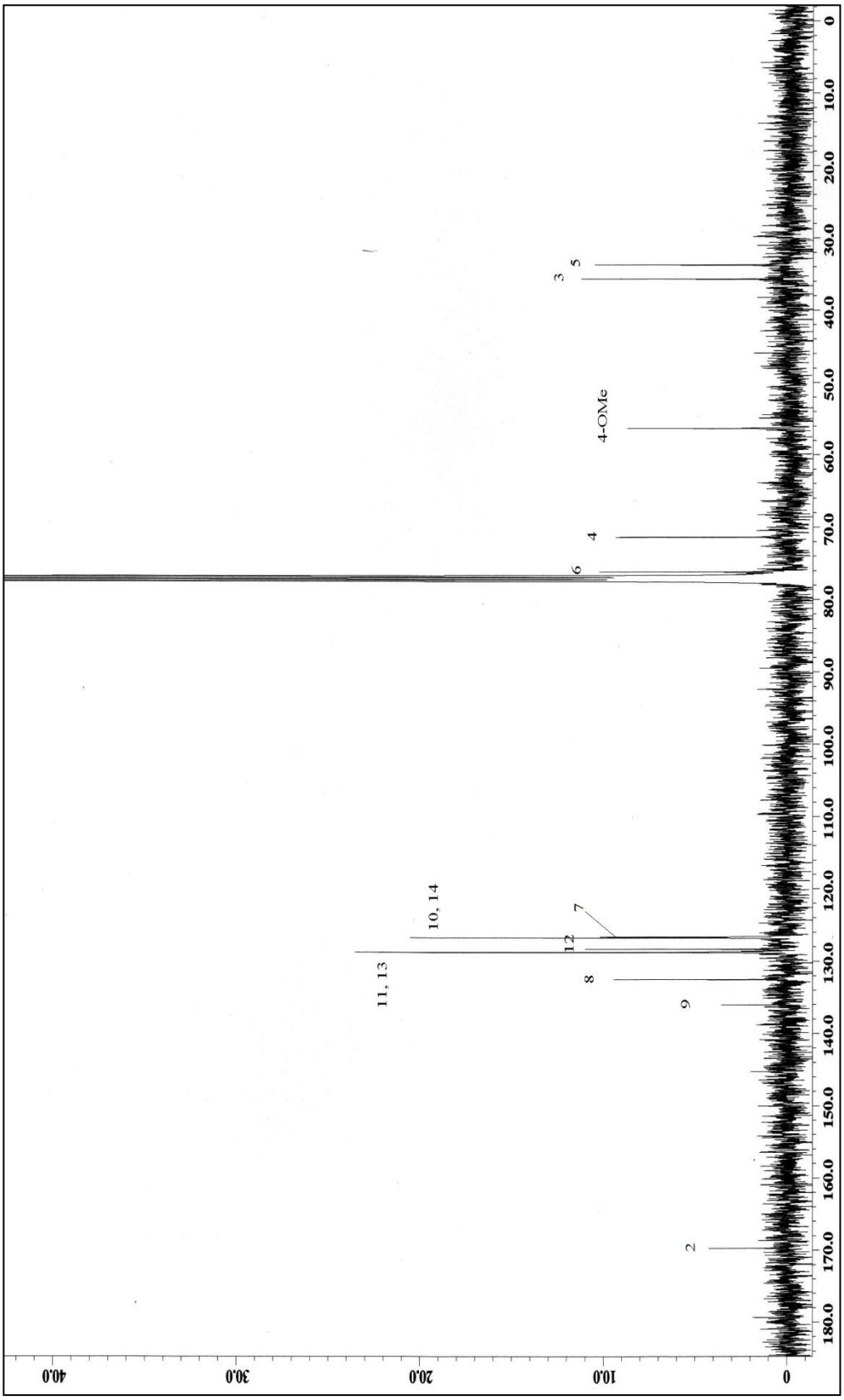


Figure 3.16: ^{13}C NMR Spectrum of **159**

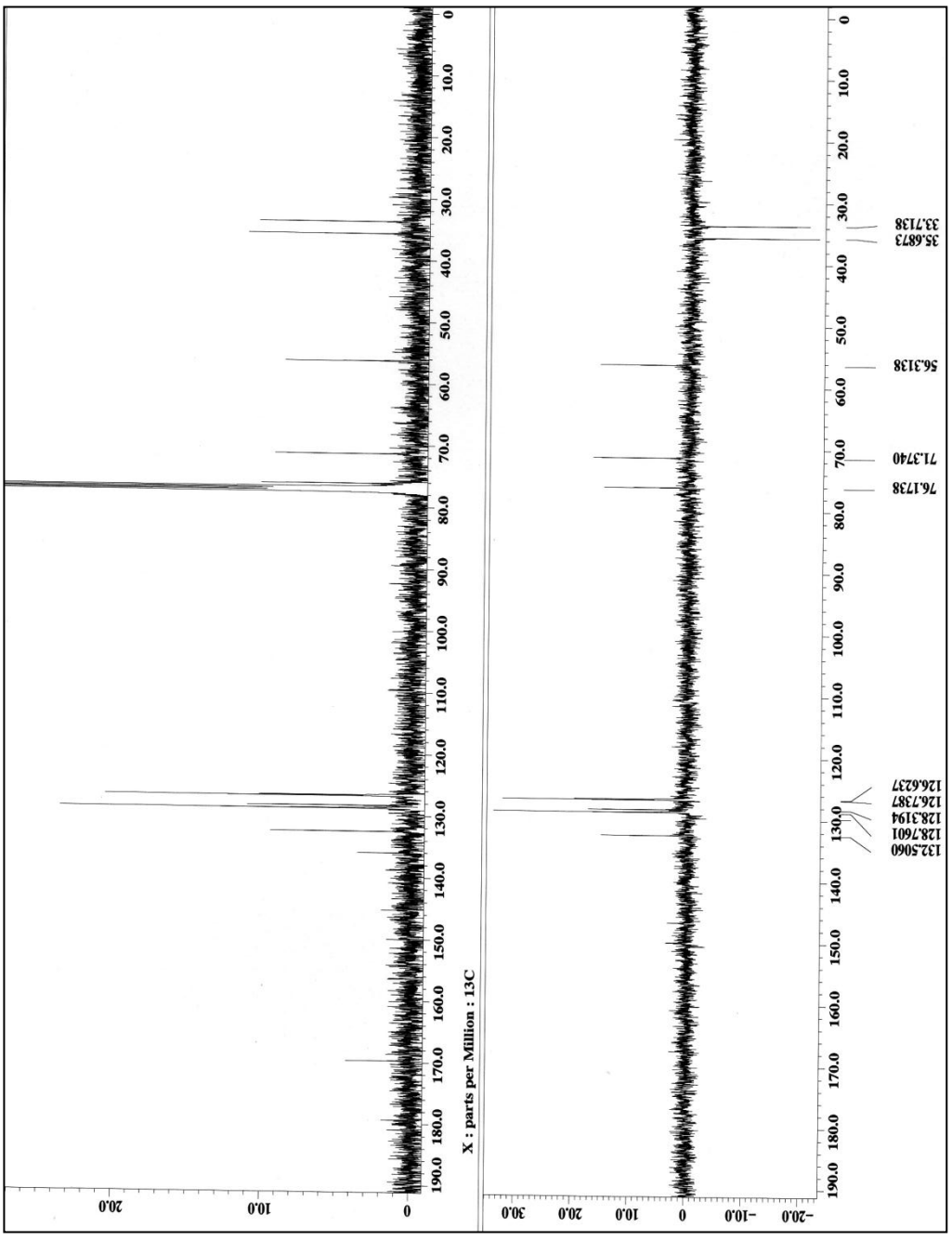


Figure 3.17: DEPT 135 Spectrum of **159**

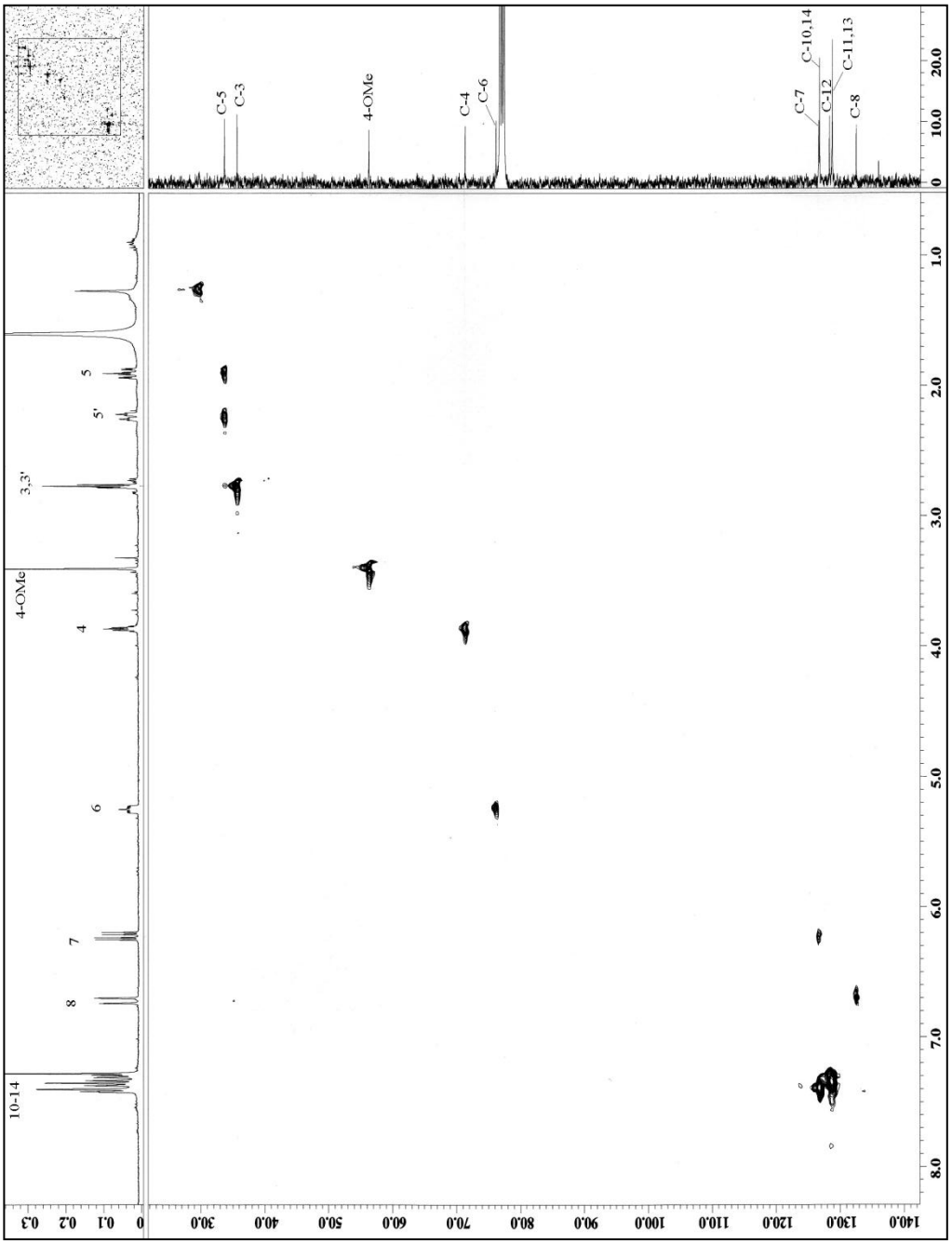


Figure 3.18: HSQC Spectrum of **159**

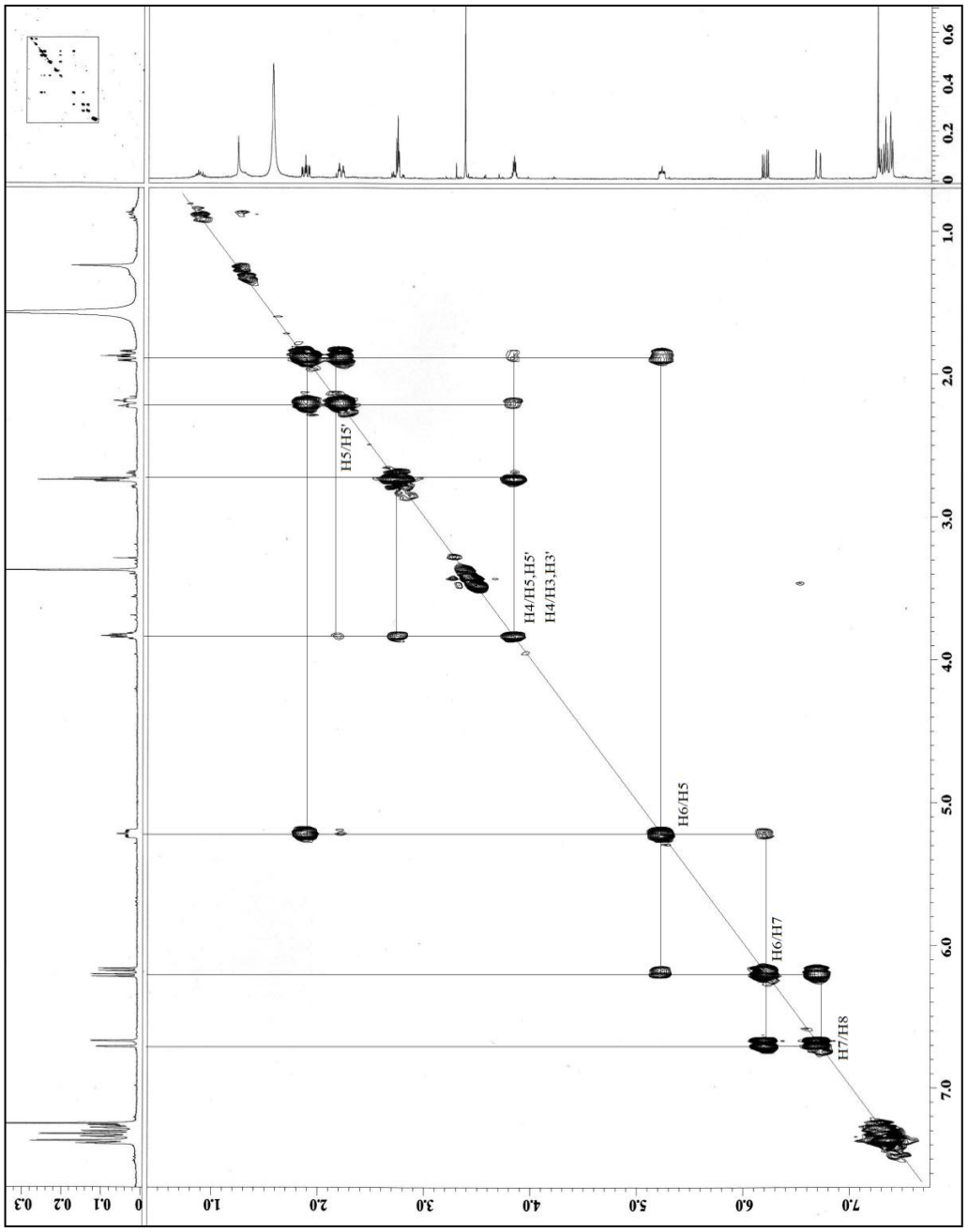


Figure 3.19: COSY Spectrum of **159**

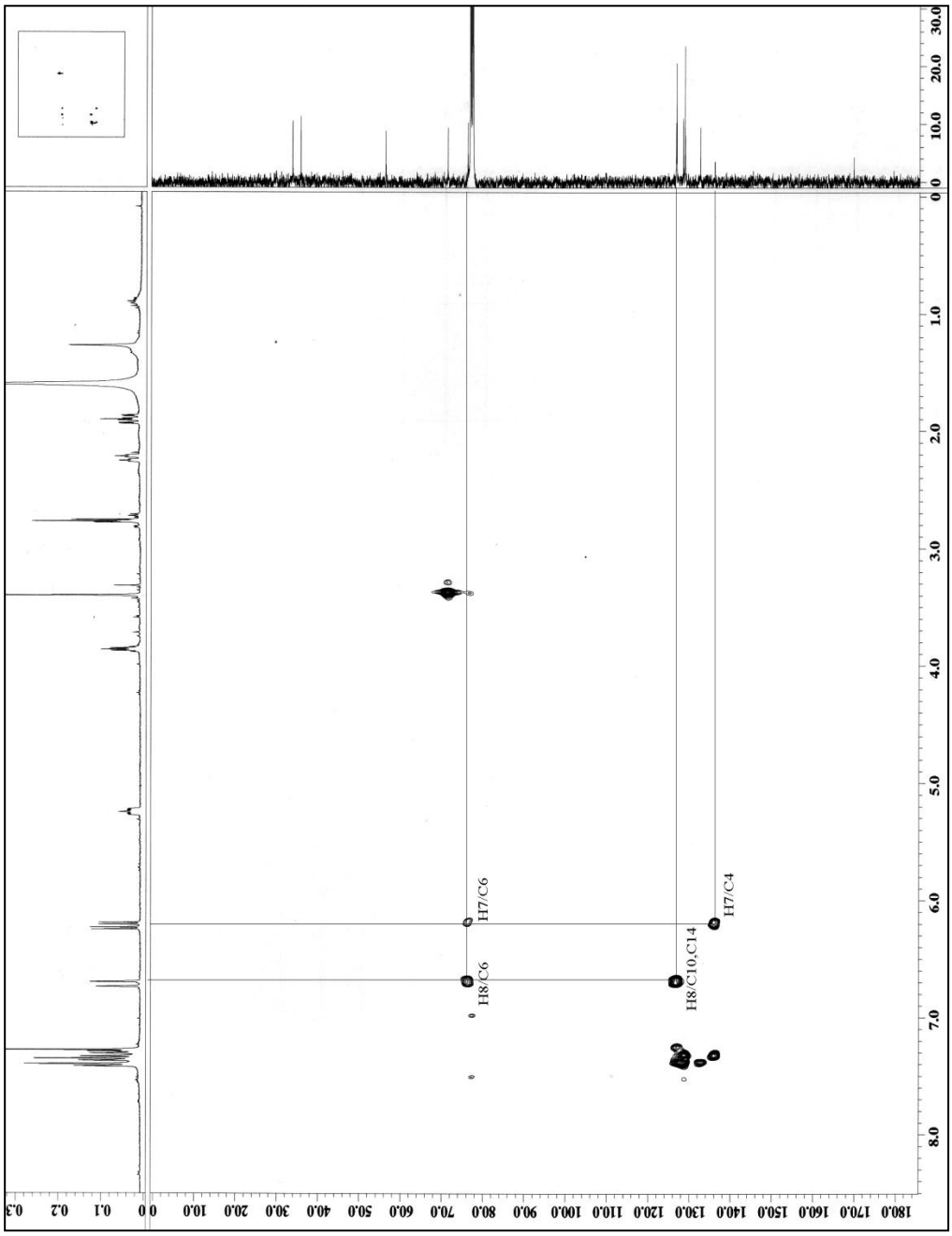
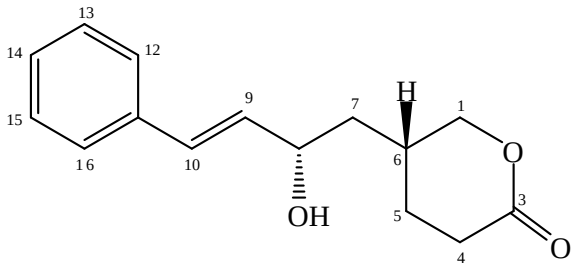


Figure 3.20: HMBC Spectrum of **159**

3.1.5 Goniomicin D **160**



160

160 was isolated as pale yellow amorphous solid. The mass spectrum showed a molecular ion peak at *m/z* 246, corresponding to a molecular formula of $C_{15}H_{18}O_3$. The IR spectrum showed strong absorptions bands of O-H stretching at 3413 cm^{-1}, C-H stretching at 2917 cm^{-1}, C=O stretching at 1662 cm^{-1} and C-O stretching at 1204 cm^{-1}. [111] The UV spectrum revealed maximum absorption at 206 and 252 nm.

The ^{1}H NMR spectrum showed the aromatic protons between δ 7.19-7.34 referring to five aromatic protons (H-12 to H-16) of a *mono*-substituted phenyl ring. Two olefinic protons peaks at δ 6.63 (*d, J*=16.0) and δ 6.20 (*dd, J*=16.0, 6.0), with a *trans* configuration belonging to H-10 and H-9 were observed. Two non-equivalent methylene protons of C-1 bearing oxygen of the lactone group appeared at δ 4.13 (*d, J*=9.2) and δ 4.64 (*d, J*=9.2) belonged to the H-1 and H-1'. The oxygen bearing proton H-8 gave a multiplet signal at δ 4.17.

The ^{13}C NMR spectrum showed 15 peaks confirmed that this compound indeed contained fifteen carbons, comprising of four methylene, nine methine and two quaternary carbons. Two olefinic carbons peak at δ 128.3 and δ 131.3 belonged to C-9 and C-10. The carbons C-1 and C-8 resonated at δ 82.9 and δ 77.3 were due to the deshielding effect by the neighbouring oxygen atom.

The COSY spectrum showed H-6/H-7 and H-7/H-8 cross peaks therefore suggesting the sequence of H-6 to H-8. The HMBC correlations of H-9, H-10 to C-8, C-

11 confirmed that the aromatic ring was connected to C-9. The correlations of H-1 to C-5, C-6, C-8 and H-5 to C-3, C-6, as well as of H-4 to C-3, C-6, C-7 supported the assignment of a lactone ring.

Therefore, **160** was identified as (*E*)-5-(2-hydroxy-4-phenylbut-3-enyl)tetrahydro-2*H*-pyran-2-one, named as goniomicin D.

Table 3.6: ^{1}H, ^{13}C and HMBC Spectral Data of **160** in $CDCl_3$

Position	**δ_C (ppm)**	**δ_H (ppm), *J*(Hz)**	**HMBC (H→C)**
1	82.9	4.13 (1H, *d*) *J*=9.2	5, 6, 8
		4.64 (1H, *d*) *J*=9.2	6, 8
2			
3	168.9		
4	37.2	2.28 (1H, *dd*) *J*=17.6, 7.8	6, 7
		2.64 (1H, *dd*) *J*=17.6, 5.5	3
5	58.9	3.82 (1H, *d*) *J*=8.7	6
		4.34 (1H, *dd*) *J*=8.7, 2.7	3, 6
6	54.1	2.98 (1H, *m*)	
7	35.6	1.74 (2H, *m*)	6, 8
8	77.3	4.17 (1H, *m*)	
9	128.3	6.20 (1H, *dd*) *J*=16.0, 6.0	8, 11
10	131.3	6.63 (1H, *d*) *J*=16.0	8, 12, 16
8-OH		6.41 (OH, *br s*)	
11	136.4		
12-16	126.6	7.19-7.34 (5H, *m*)	
	128.7		
	128.0		
	128.7		
	126.6		

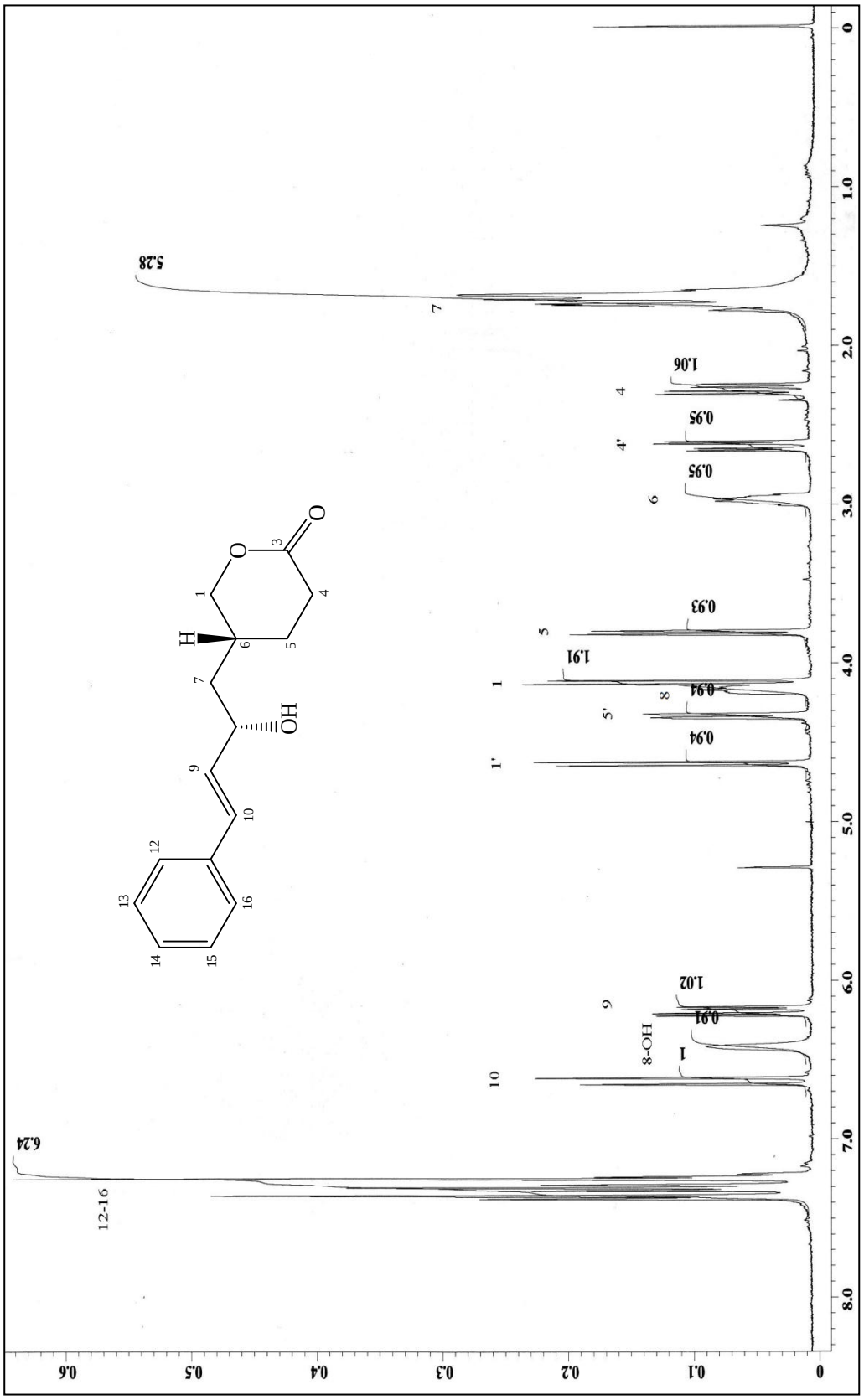


Figure 3.21: ^{1}H NMR Spectrum of **160**

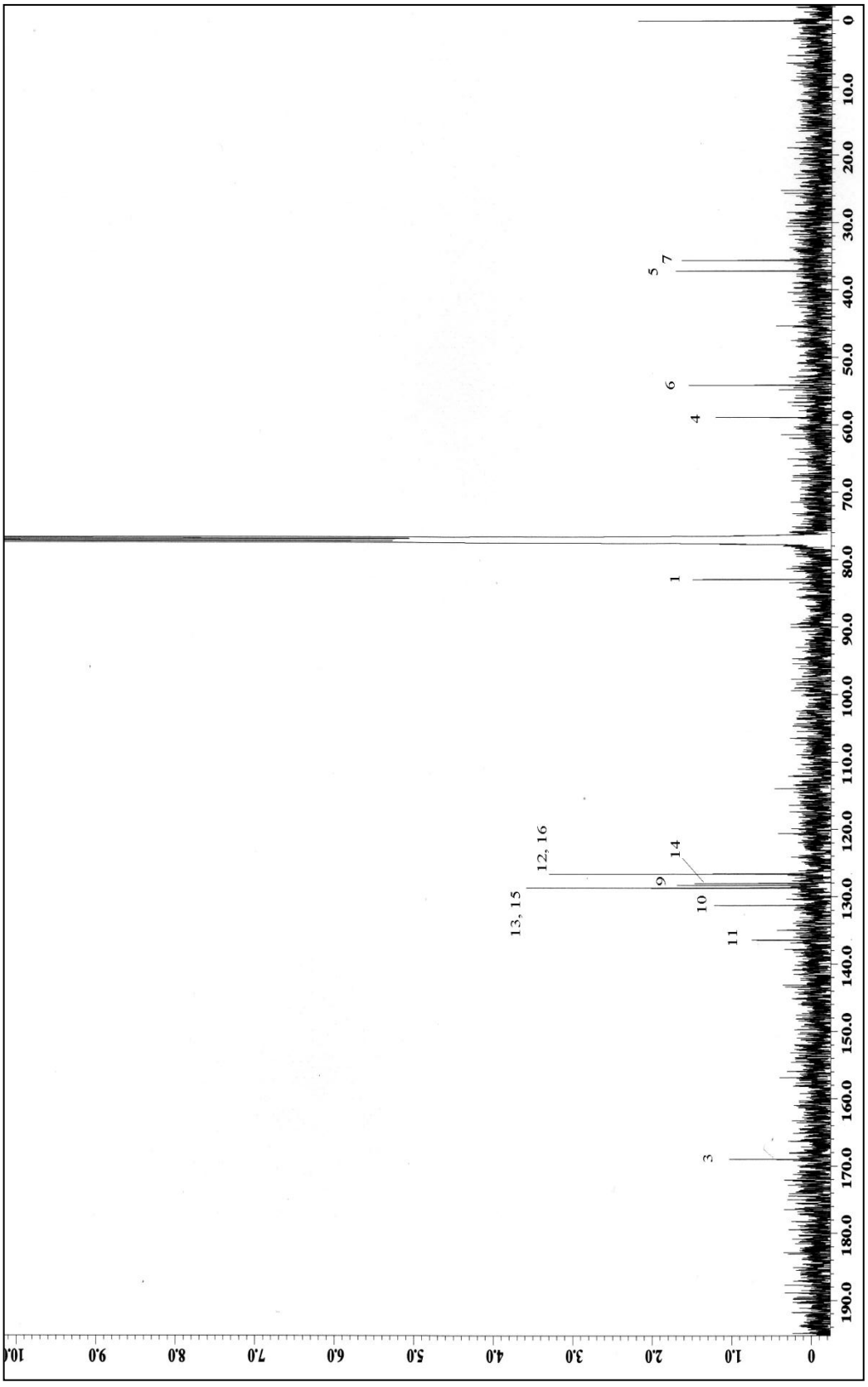


Figure 3.22: ^{13}C NMR Spectrum of **160**

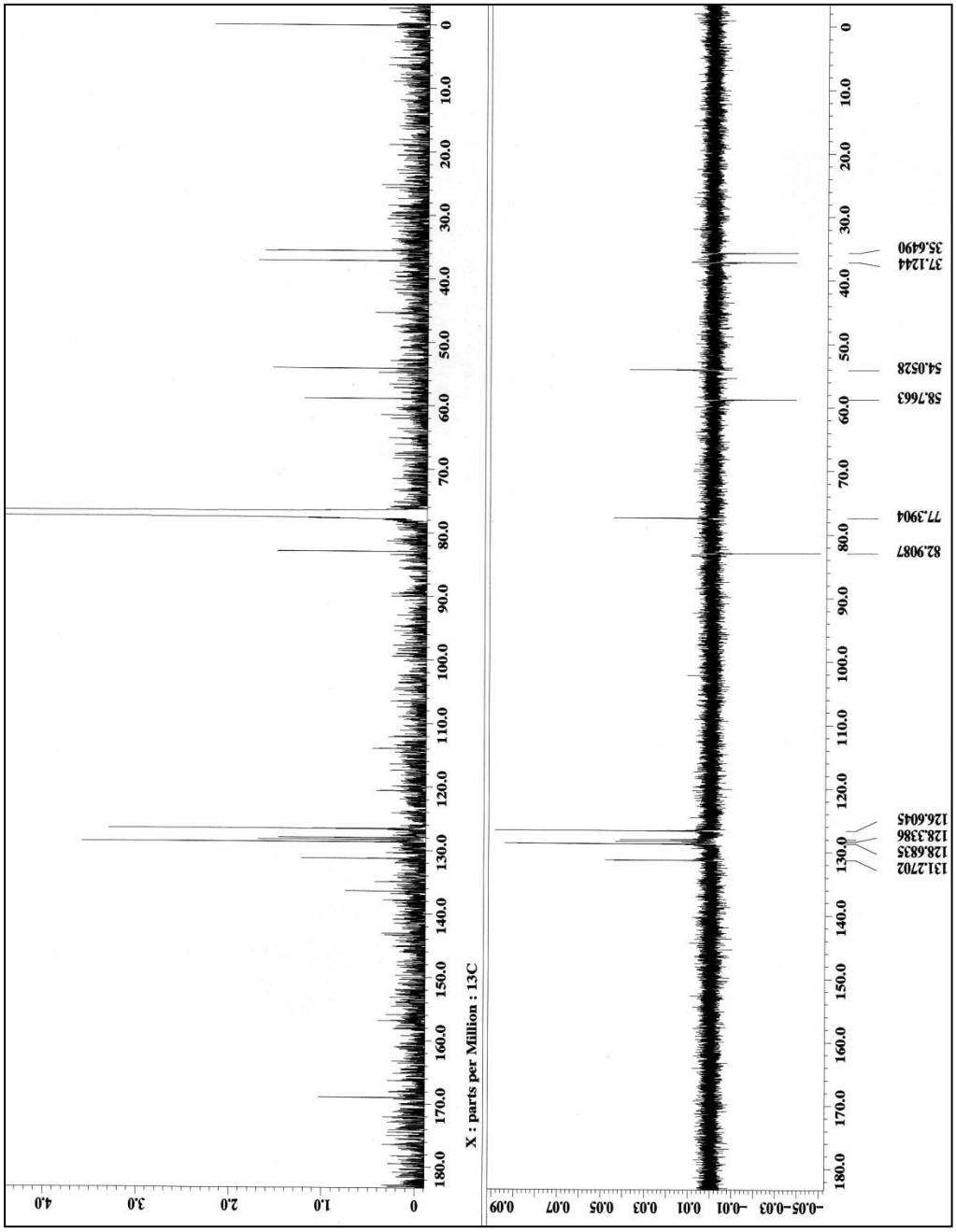


Figure 3.23: DEPT 135 Spectrum of **160**

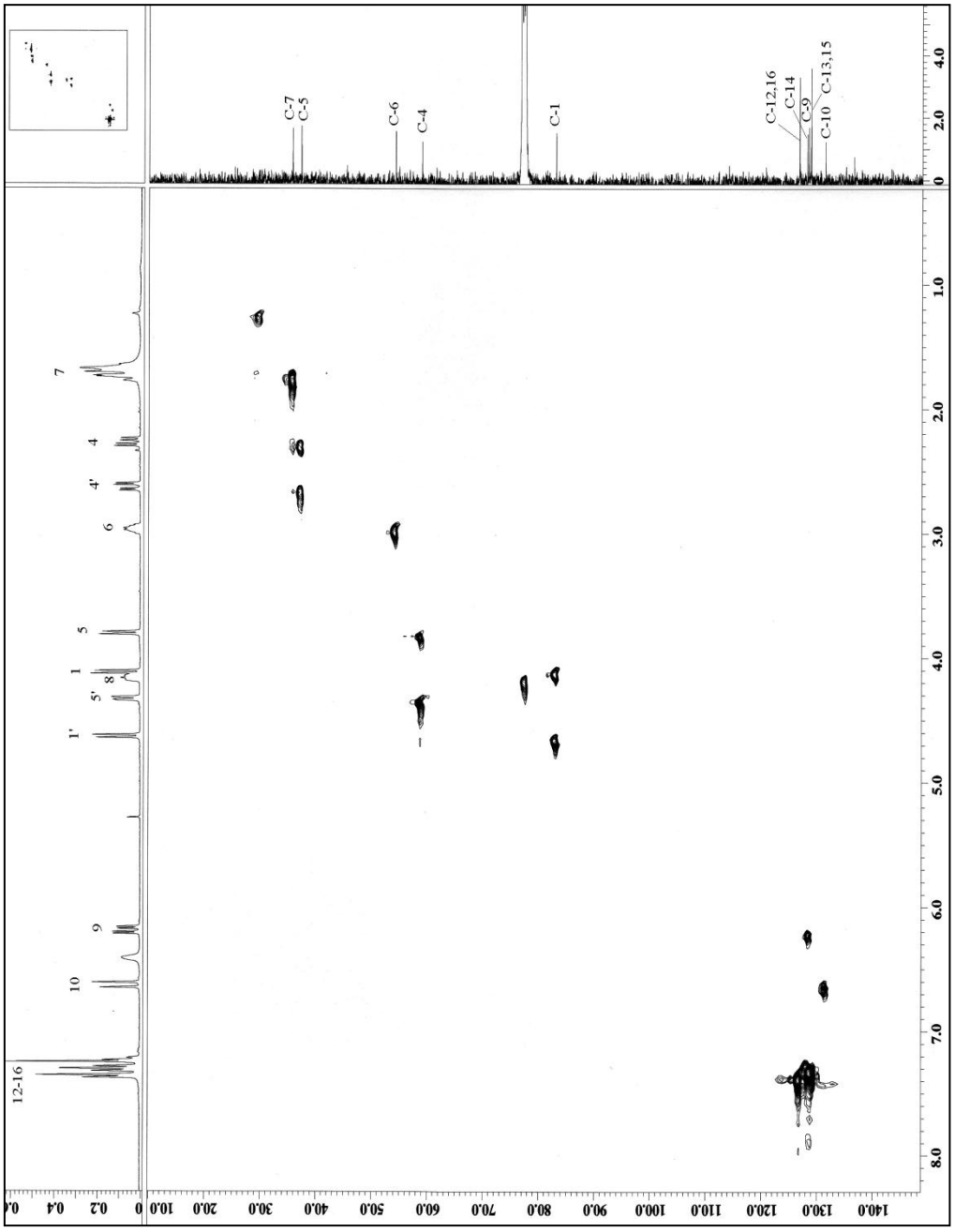


Figure 3.24: HSQC Spectrum of **160**

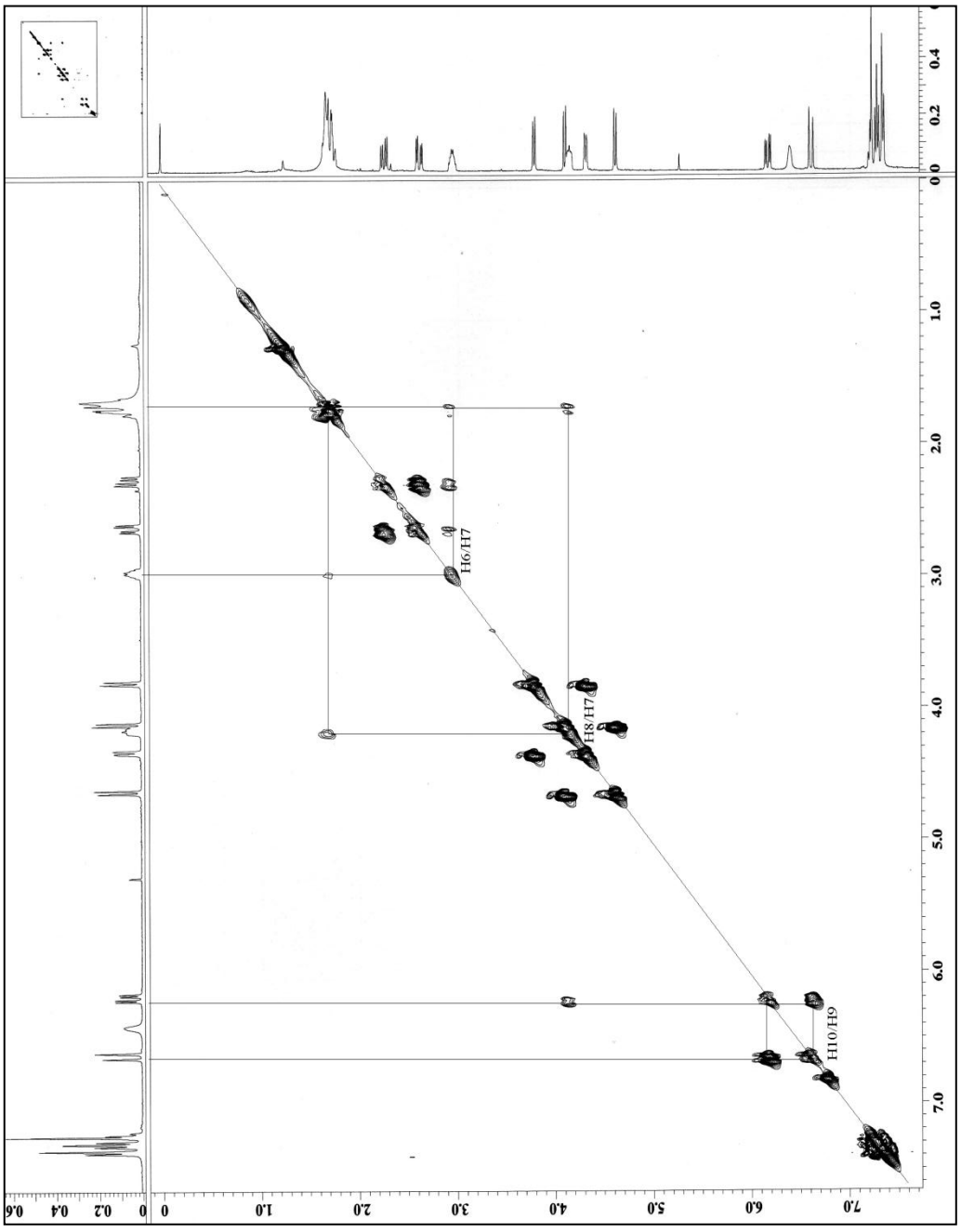


Figure 3.25: COSY Spectrum of **160**

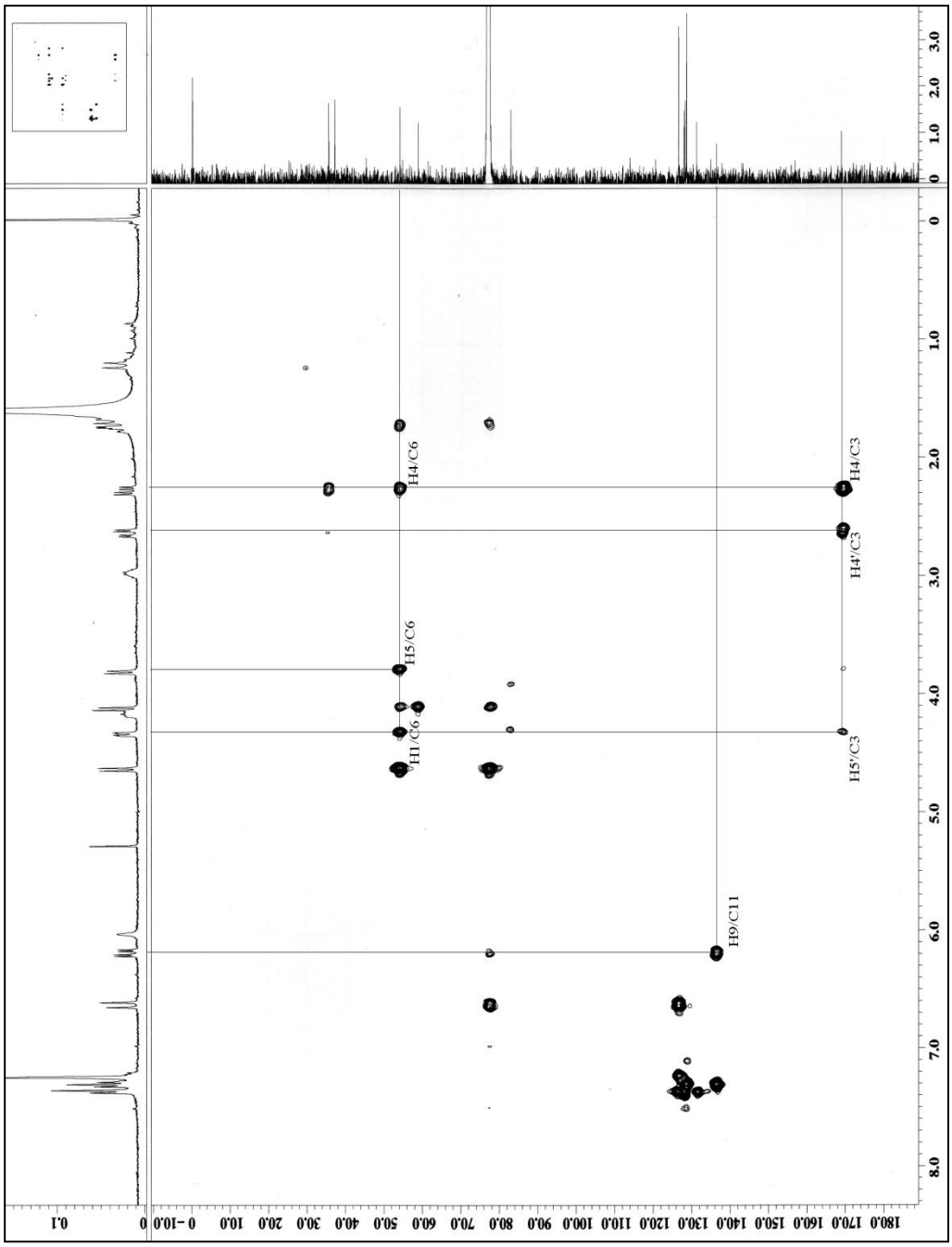
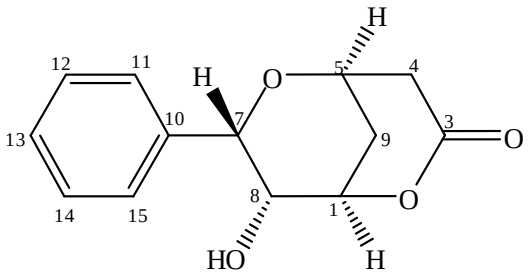


Figure 3.26: HMBC Spectrum of **160**

3.1.6 9-Deoxygoniopypyrone **15**



15

15 was isolated as white powder. The mass spectrum showed a molecular ion peak at *m/z* 234, corresponding to a molecular formula of $C_{13}H_{14}O_4$. The IR spectrum showed strong absorptions bands of O-H stretching at 3566 cm^{-1} and C=O stretching at 1720 cm^{-1}. [111] The UV spectrum with absorptions bands at 206 and 258 nm.

The ^{1}H NMR spectrum showed a multiplet δ 7.28-7.41 referring to five aromatic protons (H-11 to H-15) from a *mono*-substituted phenyl ring. Four deshielded one-proton signal at δ 4.51 (*br s*), δ 4.94 (*s*), δ 4.86 (*quin*) and δ 3.93 (*br s*) were indicative of oxygen bearing methine protons belonged to H-5, H-7, H-1 and H-8 respectively. Two protons at position 9 are non-equivalent methylene protons at δ 1.84 (*dd, J*=14.2, 3.6 Hz) and δ 2.58 (*dd, J*=14.2, 2.1 Hz).

The ^{13}C NMR spectrum showed thirteen carbons; two methylene, nine methine and two quaternary carbon. Two quaternary carbon peaks at δ 169.3 and δ 136.8 were assigned to C-3 and C-10 respectively. Four carbons, C-1, C-5, C-7 and C-8, resonated in between δ 66.2 – δ 74.8 is due to the deshielding effect by the neighbouring oxygen atom. Finally the aromatic carbon peaks showed signal at δ 126.2 attributed to the two aromatic carbons of C-11 and C-15, meanwhile the peak at δ 129.0 corresponding to the two aromatic carbons of C-12 and C-14 and *para* aromatic carbon peak appeared at δ 128.4 which was assigned for C-13. The carbonyl carbon of the lactone appeared at δ 169.3.

Comparison of the obtained spectral data with the literature values confirmed that **15** was the styryl-lactone, 9-deoxygoniopypyrone [103].

Table 3.7: ^{1}H, ^{13}C and HMBC Spectral Data of **15** in $CDCl_3$

Position	**δ_C (ppm)**	**δ_H (ppm), *J*(Hz)**	**HMBC (H→C)**
1	74.8	4.86 (1H, *quin*) *J*=2.1	
2			
3	169.3		
4	36.6	2.96 (1H, *d*) *J*=20.0	3
		2.86 (1H, *dd*) *J*=20.0, 5.5	
5	66.2	4.51 (1H, *br s*)	
6			
7	70.6	4.94 (1H, *s*)	10, 11
8	68.4	3.93 (1H, *br s*)	
9	24.1	1.84 (1H, *dd*) *J*=14.2, 3.6	
		2.58 (1H, *dd*) *J*=14.2, 2.1	
OH		-	
10	136.8		
11-15	126.2	7.28-7.41 (5H, *m*)	
	129.0		
	128.4		
	129.0		
	126.2		

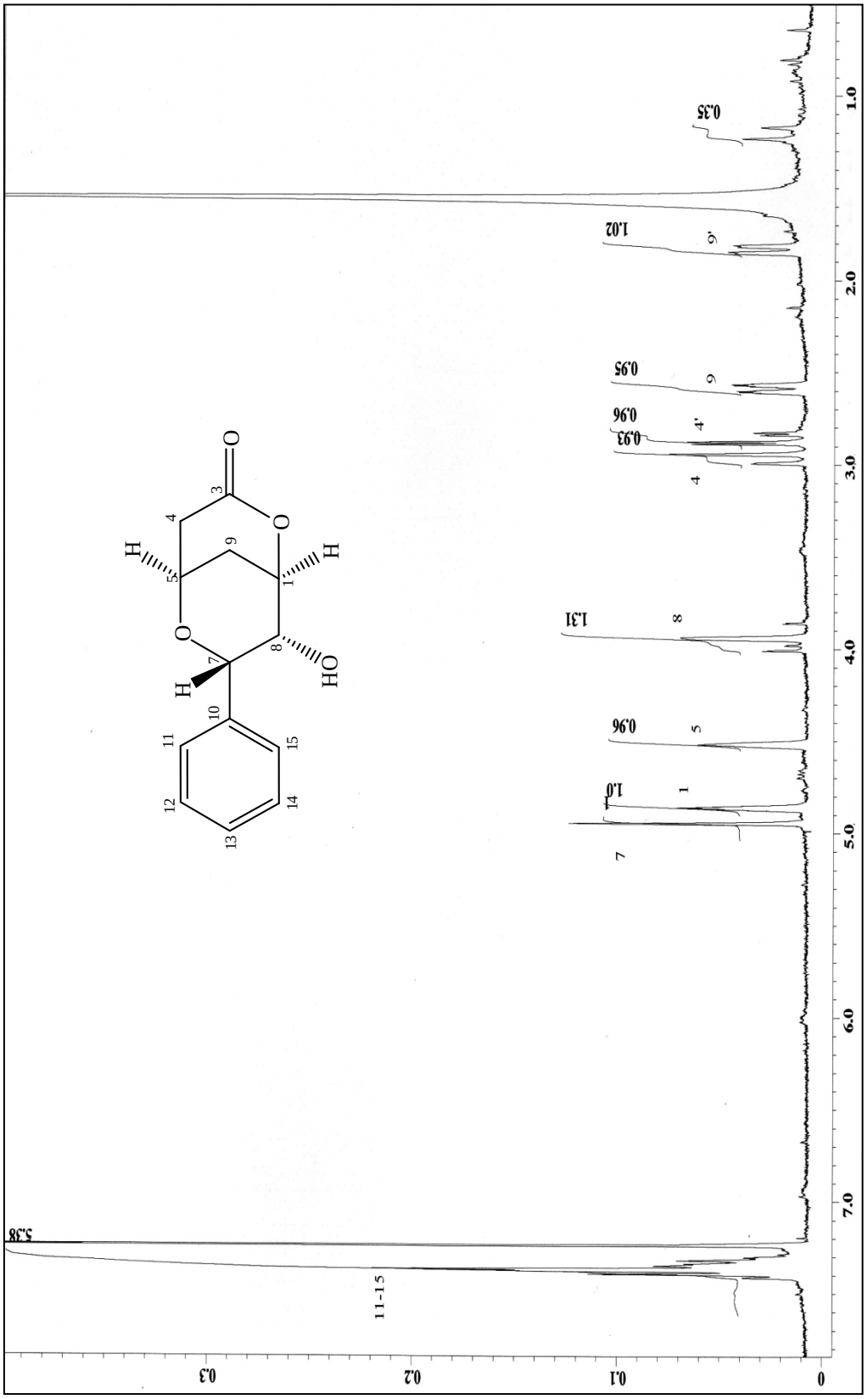


Figure 3.27: ^{1}H NMR Spectrum of **15**

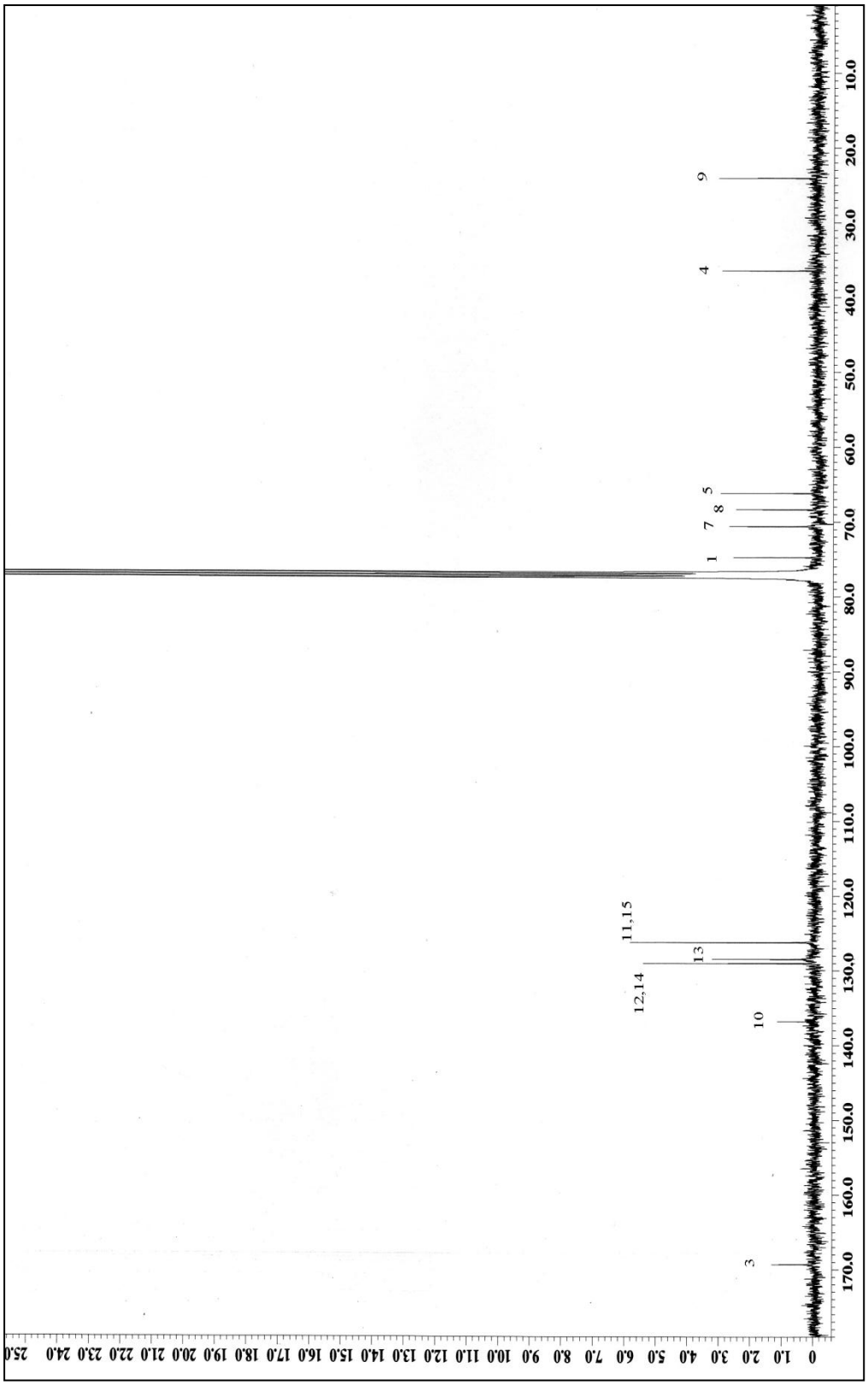
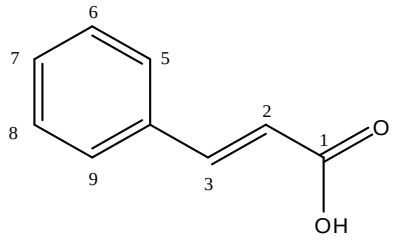


Figure 3.28: ^{13}C NMR Spectrum of **15**

3.1.7 Cinnamic acid **155**



155

155 was isolated as white amorphous solid. The mass spectrum showed molecular ion peak at m/z 147, which corresponding to a molecular formula of $C_9H_8O_2$. The UV spectrum revealed maximum wavelength at 216 and 271 nm.

The IR spectrum showed a band at 3362 cm^{-1} due to the stretching of O–H and a band at 1403 cm^{-1} due to C–O stretching. There is also conjugated C=O and C=C stretching observed at 1658 and 1601 cm^{-1}. [111]

The ^1H NMR spectrum showed the aromatic protons at δ 7.36-7.52 referring to five aromatic protons (H-5 to H-9) of a *mono*-substituted phenyl ring. Meanwhile, two olefinic proton peaks were observed at δ 6.45 (*d*, 1H, *J*=15.8), δ 7.64 (*dd*, 1H, *J*=15.8) with a *trans* configuration, attributable to H-2 and H-3 respectively.

The ^{13}C NMR spectrum showed two olefinic carbon peaks at δ 119.6 and δ 142.9 belonging to C-2 and C-3. Two quaternary carbon peaks at δ 168.0 and δ134.7 were assigned to C-1 and C-4 respectively.

Comparison of the spectral data with literature values confirmed that **155** was cinnamic acid. [112]

Table 3.8: ^{1}H, ^{13}C and HMBC Spectral Data of **155** in $CDCl_3$

Position	**δ_C (ppm)**	**δ_H (ppm), *J*(Hz)**	**HMBC (H→C)**
1	168.0		
2	119.6	6.45 (1H, *d*) *J*=15.8	1, 4
3	142.9	7.64 (1H, *d*) *J*=15.8	1, 2, 6, 8
4	134.7		
5-9	128.2	7.36-7.52 (5H, *m*)	
	129.1		
	130.3		
	129.1		
	128.2		

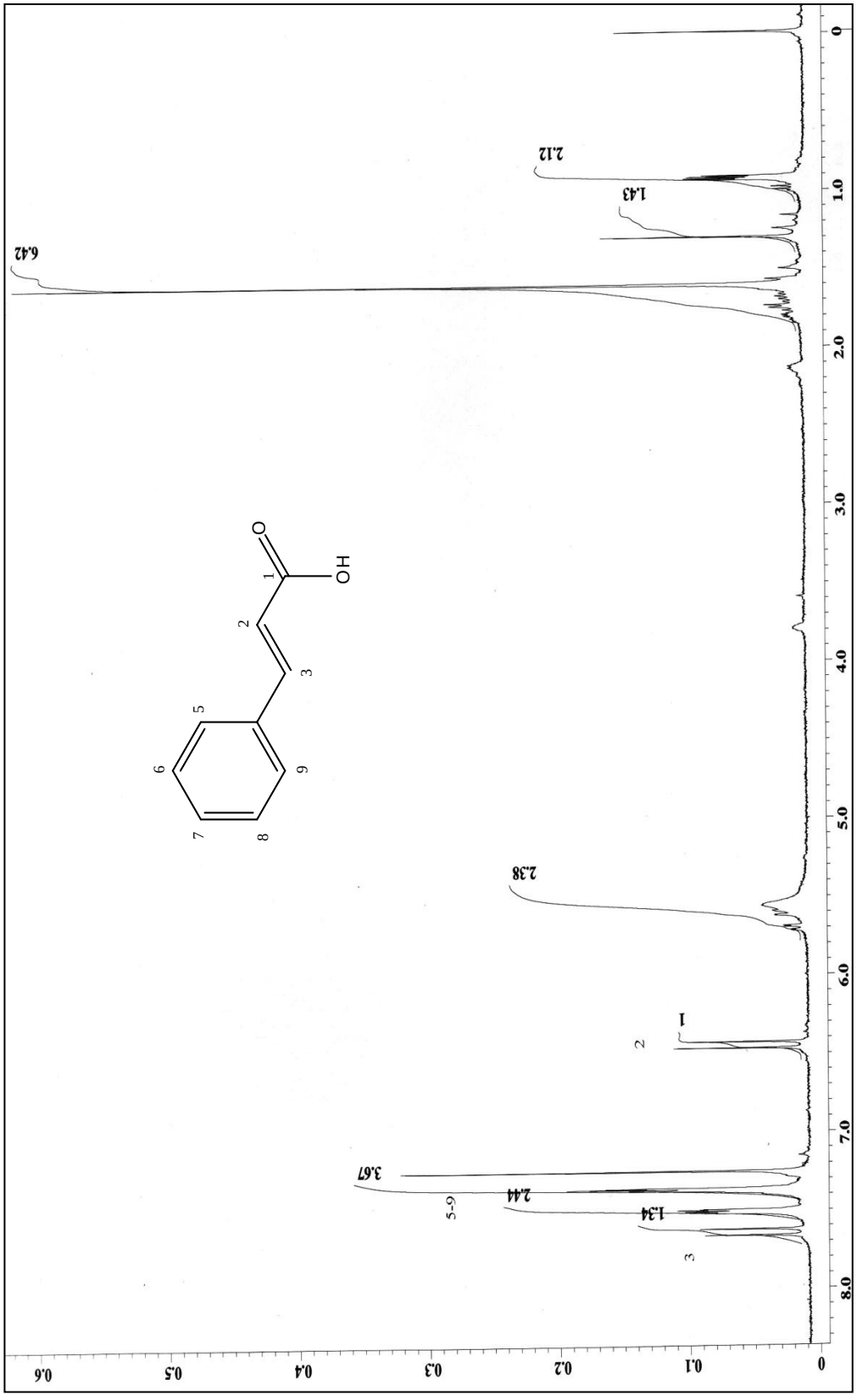


Figure 3.29: ^{1}H NMR Spectrum of **155**

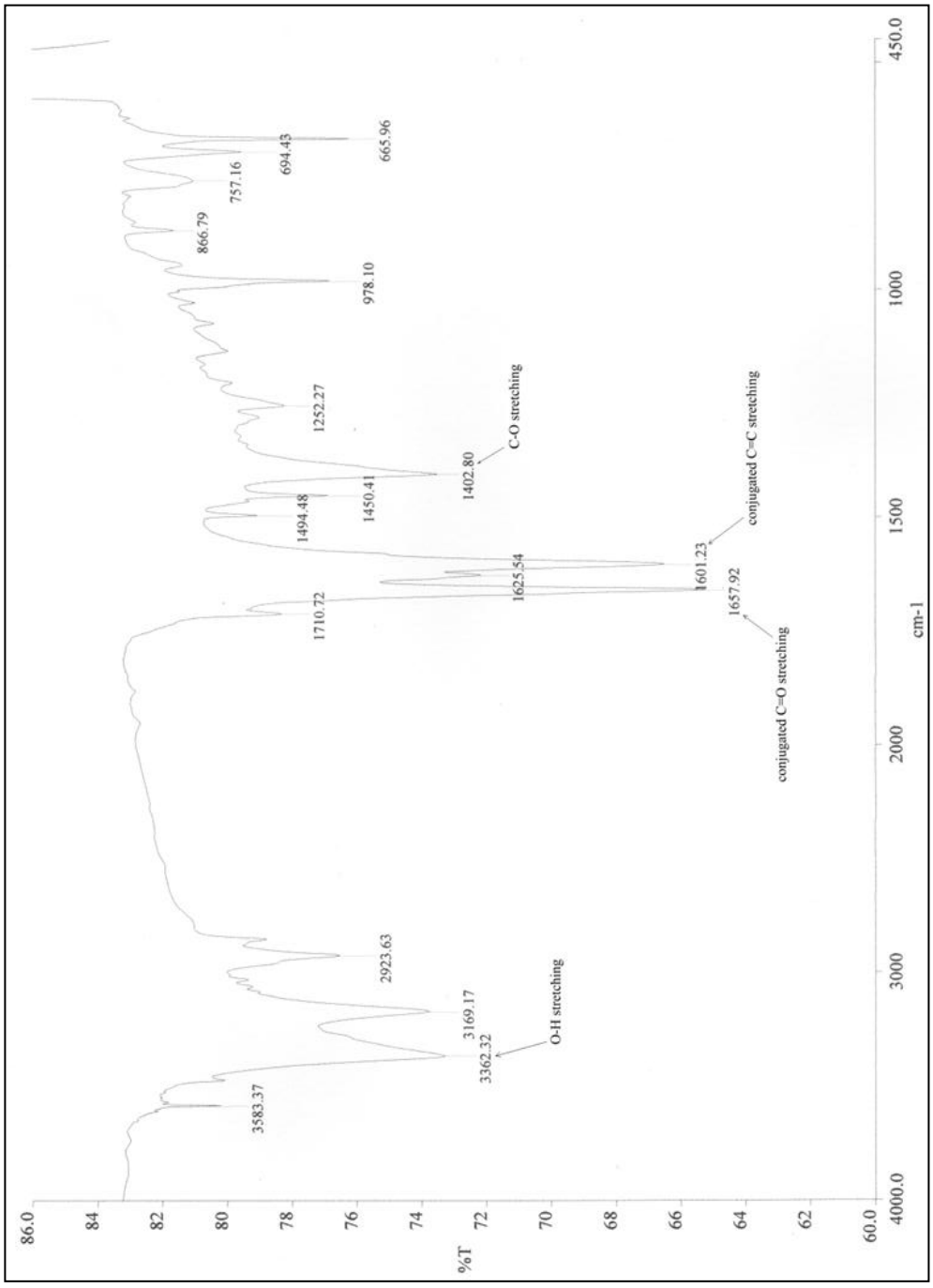
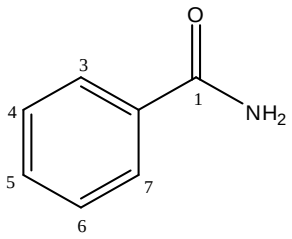


Figure 3.30: IR Spectrum of **155**

3.1.8 Benzamide **161**



161

161 was isolated as brown amorphous solid. The mass spectrum showed molecular ion peak at *m/z* 121, which corresponding to a molecular formula of C_7H_7ON. The UV spectrum revealed maximum wavelength at 207 and 252 nm.

The IR spectrum showed two bands at 3384 and 3189 cm^{-1} due to the stretching of primary amides ($–NH_2$). The presence of N–H bending at 1617 cm^{-1} and C=O stretching at 1647 cm^{-1} were also observed in the spectrum. [111]

The ^1H NMR spectrum showed the aromatic proton signals accumulated between δ 7.19-7.34 belonging to five aromatic protons of H-3 to H-7. This pattern was typical of *mono*-substituted phenyl ring. Meanwhile, the ^{13}C NMR spectrum showed a total of seven peaks, which consists of two quaternary carbon peaks and five aromatic carbon peaks centered at δ 169.5, δ 133.4, δ 127.4, δ 128.7 and δ 132.1.

Therefore, on the basis of the above results and comparison with literature values, **161** was confirmed as benzamide.

Table 3.9: ^{1}H and ^{13}C Spectral Data of **161** in $CDCl_3$

Position	**δ_C (ppm)**	**δ_H (ppm), *J*(Hz)**
1	169.5	
2	133.4	
3-7	127.4	7.42-7.80 (5H, *m*)
	128.7	
	132.1	
	128.7	
	127.4	

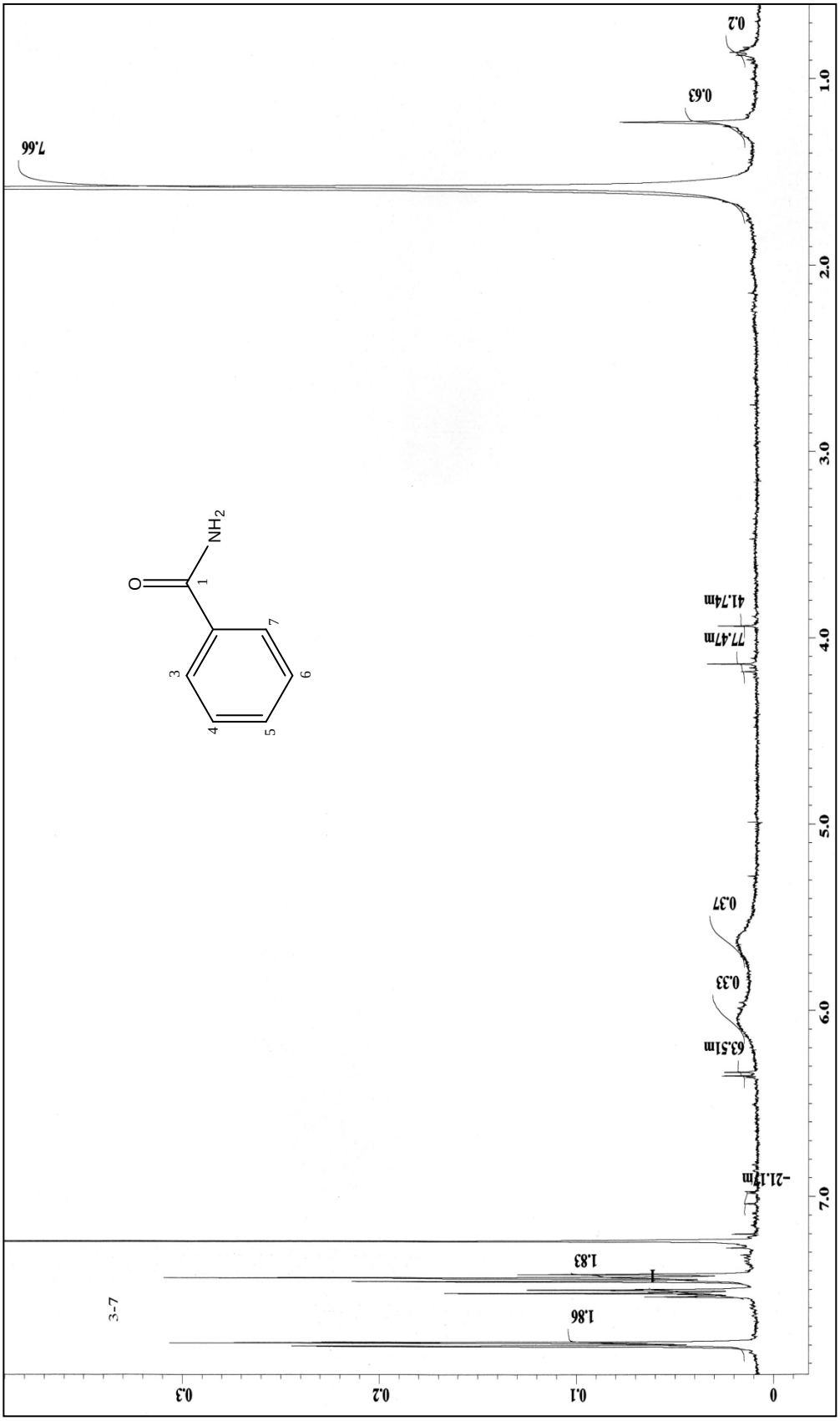


Figure 3.31: ^{1}H NMR Spectrum of **161**

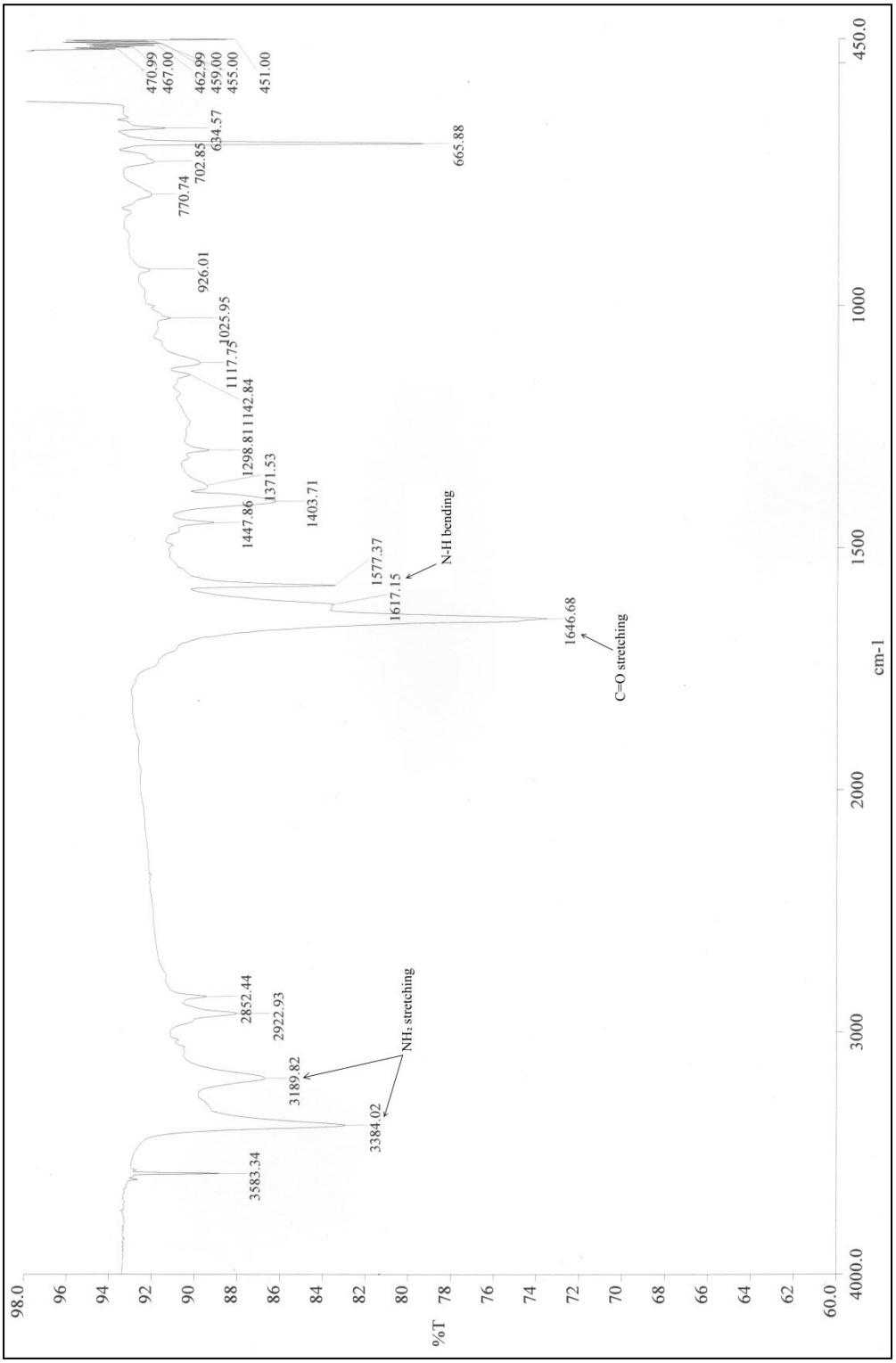
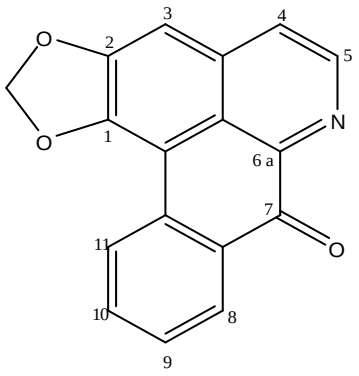


Figure 3.32: IR Spectrum of **161**

3.1.9 Liriodenine **40**



40

Liriodenine **40** was isolated as yellow amorphous solid. The mass spectrum showed a molecular ion peak at *m/z* 275, corresponding to a molecular formula of $C_{17}H_9O_3N$. Other significant fragmentations revealed by the mass spectrum were at *m/z* 247, 219, 189, 188 and 162. Fragmentation peaks at *m/z* 247 $[M\text{-}CO]^+$ and *m/z* 219 $[M\text{-}CHO]^+$ were due to the cleavage and the loss of the ketone group. The loss of the methylenedioxyl group $[M\text{-}OCH_2O]^+$ give the peak at *m/z* 189.

This alkaloid exhibited a yellow fluorescent in UV light which indicated the presence of an oxoaporphine chromophore. [113] The IR spectrum exhibited a strong peak due to the C=O stretching at 1728 cm^{-1} signified the occurrence of a highly conjugated chromophore [114,115] with a ketone group enwrapped within the system.

The ^1H NMR spectrum revealed the characteristic AB *dd*, typical of an aporphinic H-4 and H-5 coupling pattern [113]. H-4 and H-5 resonated at δ 7.79 and δ 8.89 respectively with a coupling constant of 5.06 Hz each. A singlet was observed at δ 7.21 attributed to H-3. The methylenedioxyl group attached to the C-1 and C-2 gave a singlet of two protons at δ 6.37.

Moreover, two sets of doublet at δ 8.67 (*J*=7.8 Hz) and δ 8.59 (*J*=7.76 Hz) corresponding to two protons at ring D. The former pair was assigned to H-11 and the latter was assigned to H-8. H-11 usually resonated more downfield than the other

aromatic protons due to deshielding effect of the facing ring A. Two sets of triplet with *J*=7.2 at δ 7.78 and δ 7.58 were observed, which can be attributed to H-10 and H-9.

Comparison of the obtained spectral data with the literature values confirmed that **40** was the oxoaporphine liriodenine [114,115].

Table 3.10: ^{1}H and ^{13}C Spectral Data of **40** in $CDCl_3$

Position	**δ_C (ppm)**	**δ_H (ppm), *J*(Hz)**
1	163.2	
2	165.3	
3	105.6	7.21 (1H, *s*)
3a	146.2	
4	129.0	7.79 (1H, *d*) *J*=5.06
5	140.0	8.89 (1H, *d*) *J*=5.06
6a	161.4	
7	179.9	
7a	129.8	
8	131.2	8.59 (1H, *d*) *J*=7.8
9	132.4	7.58 (1H, *t*) *J*= 7.2
10	135.4	7.78 (1H, *t*) *J*= 7.2
11	130.4	8.67 (1H, *d*) *J*=7.8
11a	152.2	
1a	124.5	
1b	139.6	
O-CH_2-O	107.6	6.37 (*s*)

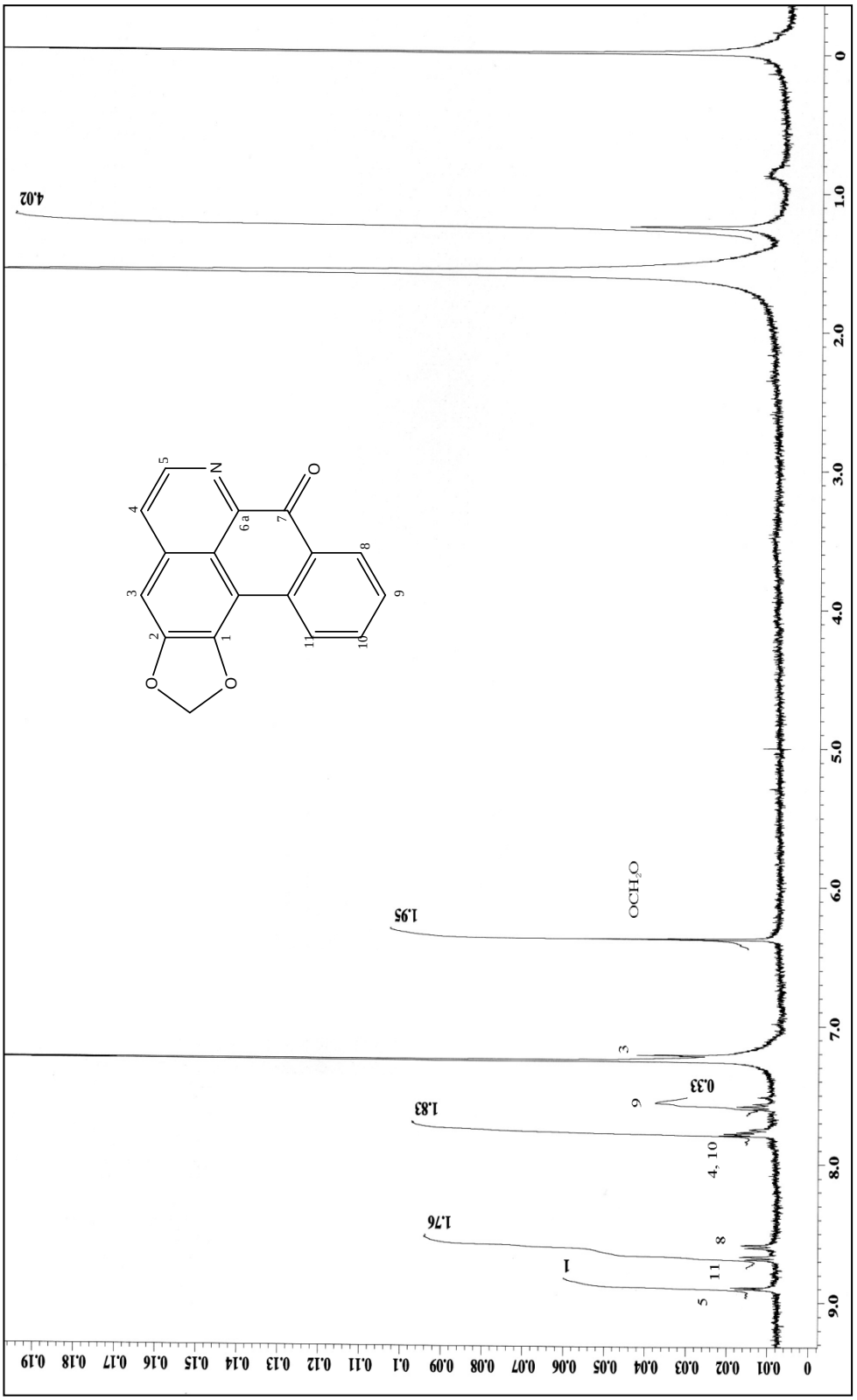
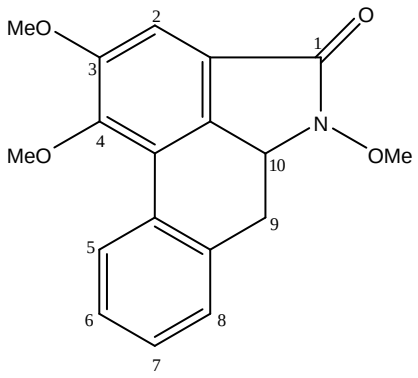


Figure 3.33: ^{1}H NMR Spectrum of **40**

3.1.10 Tapisoidin **162**



162

162 was isolated as brown amorphous solid. The mass spectrum showed a molecular ion peak at *m/z* 311, corresponding to a molecular formula of $C_{18}H_{17}O_4N$. The IR spectrum showed strong absorption peaks of C=O group at 1715 cm^{-1}. The UV spectrum showed absorption bands at 209, 251, 277 and 322 nm, indicating the presence of an aristolactam basic structure. [116]

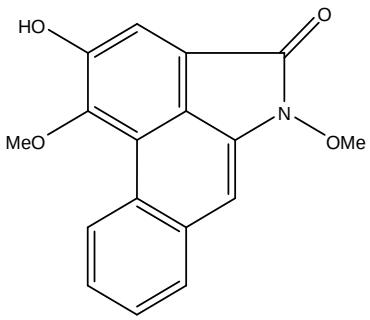
The ^{1}H NMR spectrum showed three distinct methoxyl singlets at δ 3.96 (*s*), δ 4.00 (*s*) and δ 3.89 (*s*). Those methoxyls were attached to nitrogen, C-3 and C-4 respectively. The positioning of the methyl groups were established from the NOESY spectrum which showed cross peaks between H-3 and the methoxyl protons attached to C-2. In addition, the latter showed correlations with the methoxyl protons of C-1. The isolated Pproton, H-3, appeared as a singlet at signal at δ 7.23 (*s*) belonged to an isolated aromatic proton at H-23. All H-5a, H-6a and H-6b resonated as doublet of doublets d at δ 4.60, δ 2.73, δ 3.49 indicating that C-5a and C-6 are hydrogenated. To the knowledge of the authors, this is the first occurrence of a 5a,6-dihydroaristolactam alkaloid.

Moreover, two sets of doublet of doublets at δ 8.35 (*J*=8.2, 1.4 Hz) and δ 7.35 (*J*=7.9, 1.4 Hz) corresponded to two protons in ring D. The former pair was assigned to H-5 and the latter was assigned to H-8. H-5 usually resonated more downfield than the

other aromatic protons due to the deshielding effect of the facing ring A. Two sets of doublet at δ 7.40 and δ 7.44 were observed, which could be attributed to H-6 and H-7.

The HMBC spectrum showed proton H-2 correlated to C-1, C-3 and C-10a; H-6 to C-8; and from H-9 and H-10 to C-5a confirmed the proposed structure of this compound.

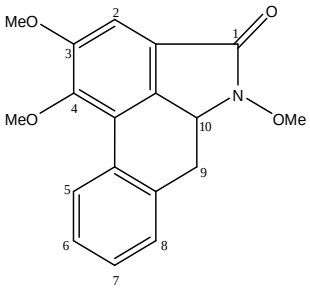
In general, aristolactam have no *N*-oxy functional group and the aromatic methoxyl carbon signals are located at δ 55.0-60.0. In the first report of a naturally occurring *N*-oxygenated methoxy aristolactam (Piperlactam S **163**), the carbon signal of MeO-N is at δ 64.5. [117]

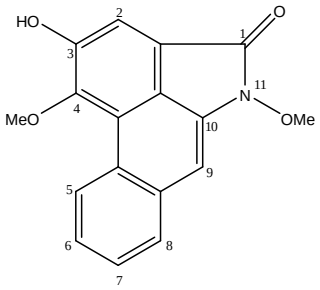


163

Comparison with the spectral data of Piperlactam S **163** (Table 3.11) with **162** confirmed that both alkaloids posses the same skeleton; *N*-oxygenated methoxy aristolactam.

Table 3.11: ^{1}H and ^{13}C NMR Spectral Data of **162** and Piperlactam S in $CDCl_3$





Position	**162**		**Piperlactam S** [48]	
	δ_C	**δ_H (ppm), *J*(Hz)**	**δ_C**	**δ_H (ppm), *J*(Hz)**
1	167.9		162.1	
2	105.9	7.23 (1H, *s*)	114.1	7.66 (1H, *s*)
2a	123.1		118.0	
3	150.5		152.4	
4	155.7		149.4	
4a	123.8		120.3	
5	127.7	8.35 (1H, *dd*) *J*=7.9, 1.4	126.9	9.14 (1H, *m*)
5a	135.1		126.4	
6	127.9	7.44 (1H, *br d*) *J*=7.9	126.0	7.67 (2H, *m*)
7	128.7	7.40 (1H, *br t*) *J*=7.9	127.6	
8	130.0	7.35 (1H, *dd*) *J*=7.9, 1.4	129.3	8.02 (1H, *m*)
8a	131.3		131.9	
9	34.8	2.73(1H, *t*) *J*=14.0 3.49 (1H, *dd*) *J*=14.0, 6.0	102.8	7.39 (1H, *s*)
10	57.3	4.60 (1H, *dd*) *J*=14.0, 6.0	134.1	
10a	134.4		117.1	
N-OCH_3	**63.7**	**3.96 (3H, *s*)**	**64.5**	**4.06 (3H, *s*)**
3-OCH_3	59.7	3.89 (3H, *s*)	3-OH	10.44 (1H, *s*)
4-OCH_3	56.0	4.00 (3H, *s*)	59.6	4.03 (3H, *s*)

Therefore, **162** was identified as 1,2,5-trimethoxy-5a,6-dihydrodibenzo[*cd*,*f*]indol-4(*5H*)-one which is a new alkaloid named as tapisoidin.

Table 3.12: ^{1}H, ^{13}C and HMBC Spectral Data of **162** in $CDCl_3$

Position	**δ_C (ppm)**	**δ_H (ppm), *J*(Hz)**	**HMBC (H→C)**
1	167.91		
2	105.86	7.23 (1H, *s*)	1, 3, 4, 10a
2a	123.09		
3	150.47		
4	155.66		
4a	123.82		
5	127.67	8.35 (1H, *dd*) *J*=7.9, 1.4	2a, 7, 10a
5a	135.07		
6	127.91	7.44 (1H, *br d*) *J*=7.9	8
7	128.65	7.40 (1H, *br t*) *J*=7.9	8a
8	129.99	7.35 (1H, *dd*) *J*=7.9, 1.4	5a
8a	131.27		
9	34.78	2.73(1H, *t*) *J*=14.0	5a, 10
		3.49 (1H, *dd*) *J*=14.0, 6.0	5a, 8a, 10
10	57.27	4.60 (1H, *dd*) *J*=14.0, 6.0	5a
10a	134.43		
N-OCH_3	63.71	3.96 (3H, *s*)	
3-OCH_3	59.68	3.89 (3H, *s*)	3
4-OCH_3	55.99	4.00 (3H, *s*)	4

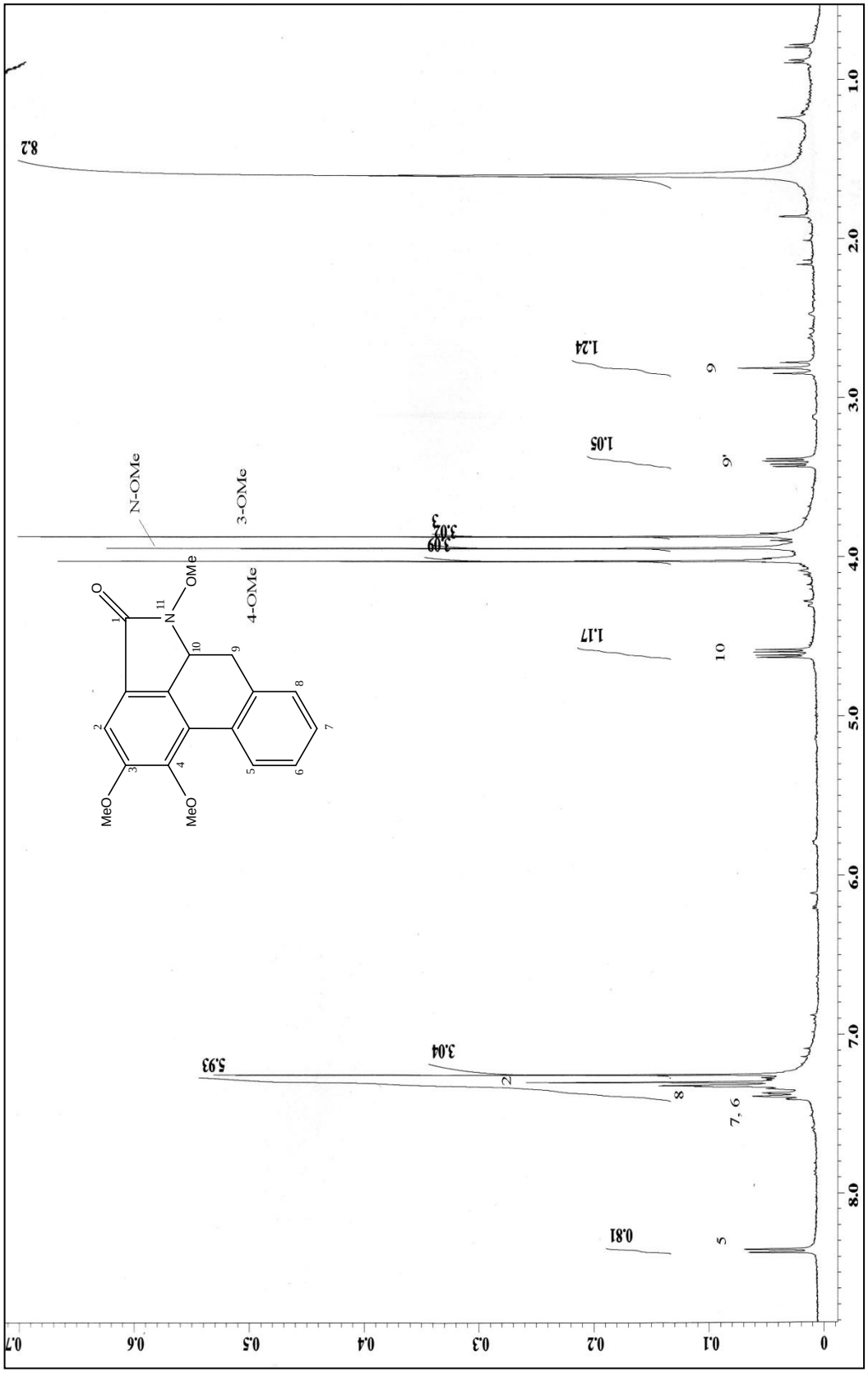


Figure 3.34: ^{1}H NMR Spectrum of **162**

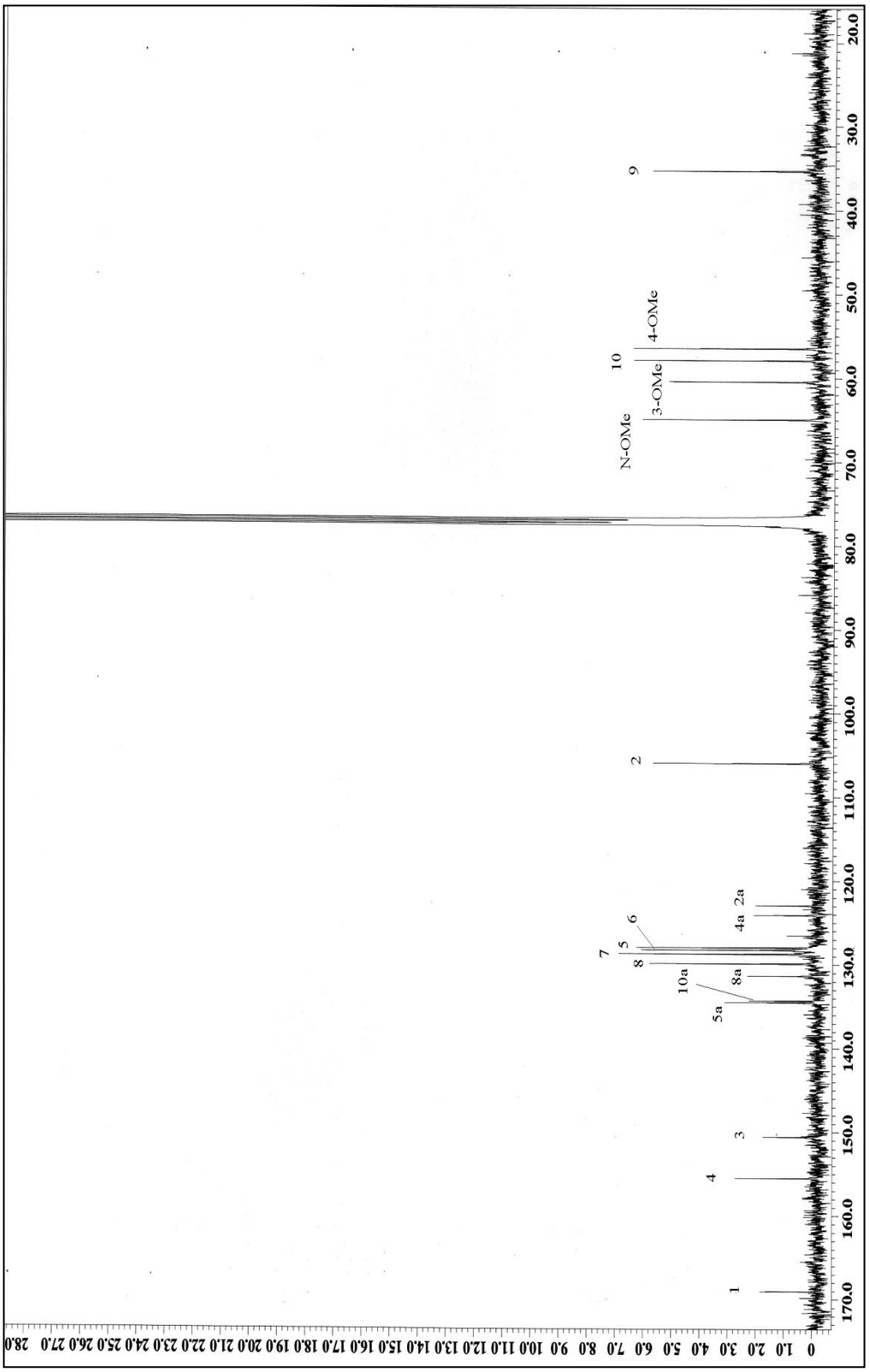


Figure 3.35: ^{13}C NMR Spectrum of **162**

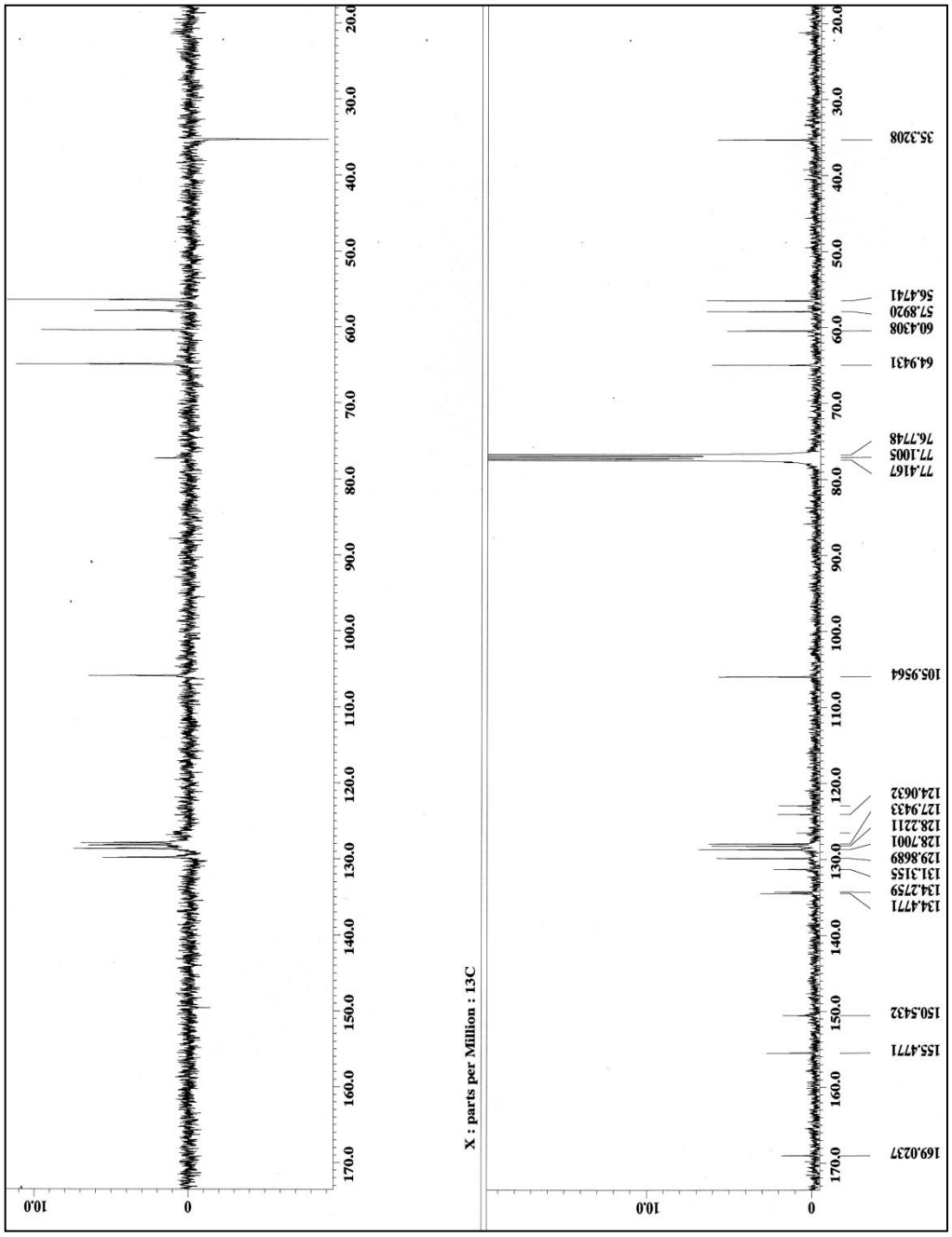


Figure 3.36: DEPT 135 Spectrum of **162**

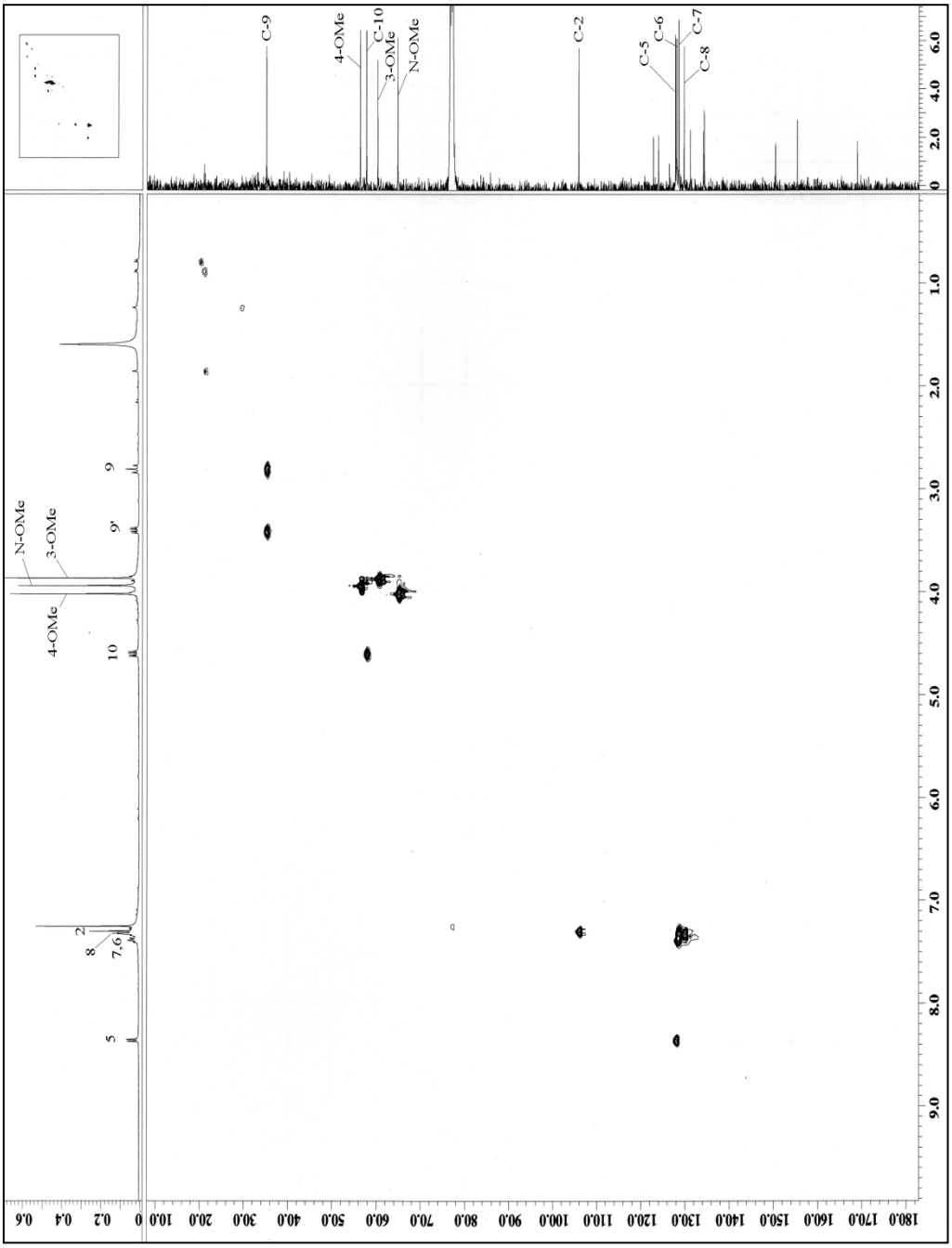


Figure 3.37: HSQC Spectrum of **162**

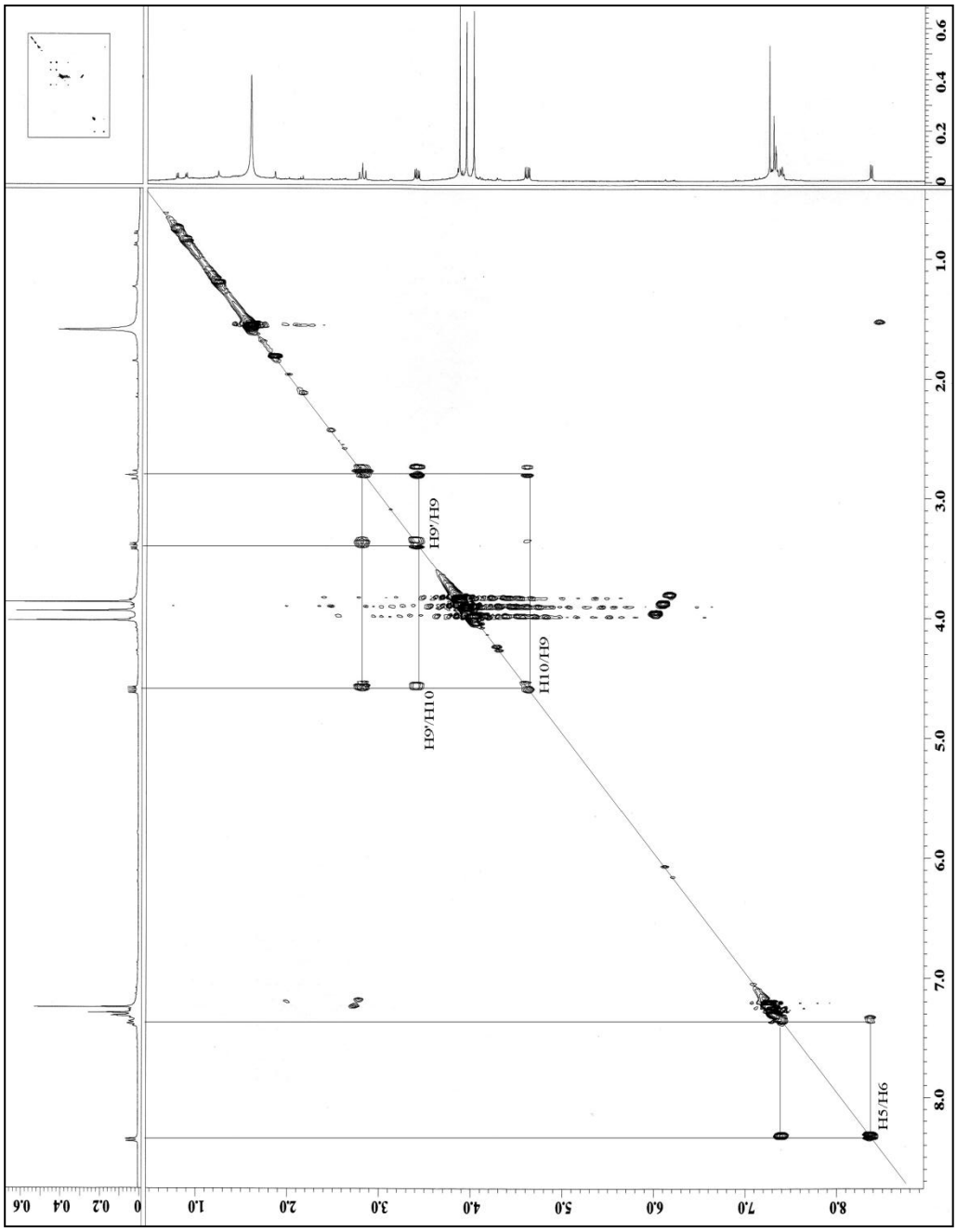


Figure 3.38: COSY Spectrum of **162**

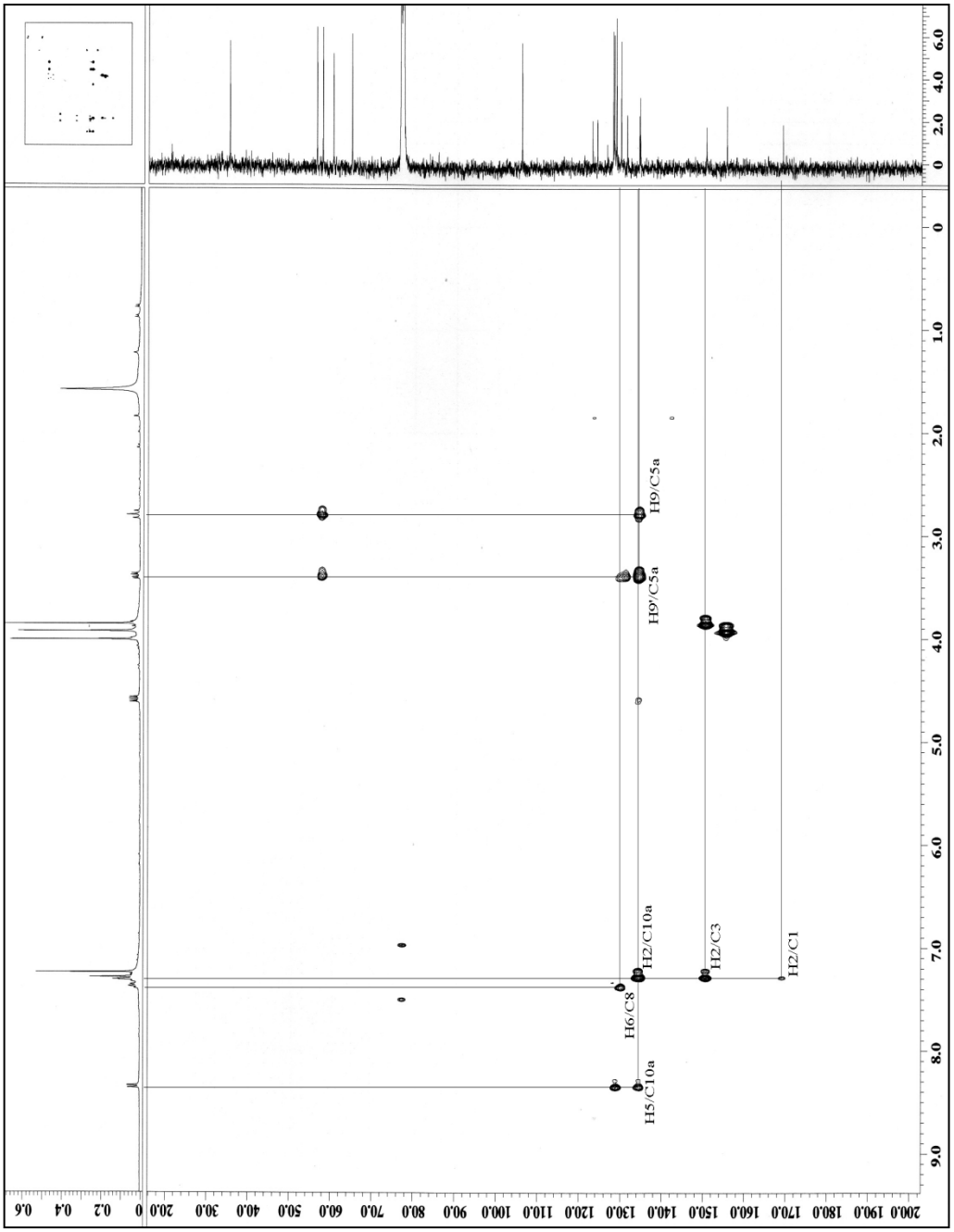
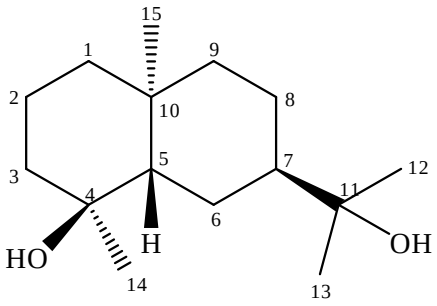


Figure 3.39: HMBC Spectrum of **162**

3.1.11 Pterodondiol **164**



164

Pterodondiol **164** was isolated as colourless needles (mp 110-112°C). The mass spectrum showed a fragmentation peak at *m/z* 222, corresponding to a molecular formula of $C_{15}H_{28}O_2$. This fragment arises from the loss of one water molecule from pterodondiol. The IR spectrum showed strong absorptions bands of O-H stretching at 3391 cm^{-1} and alkyl C-H stretching at 2971 cm^{-1} and 2930 cm^{-1}. [111]

^{1}H NMR spectrum showed three single peaks at δ 1.20, δ 1.11 and δ 0.86 corresponding to four methyl groups at position 12-15. Methyl group at position 12 and 13 existed in the same peak at δ 1.20. Twelve protons of 4 methyl groups thus suggesting that compound K could be a sesquiterpene.

The ^{13}C NMR spectrum showed fifteen carbons; four methyl, six methylene, two methine and three quaternary carbon. Three quaternary carbon peaks at δ 73.1, δ 34.6 and δ 72.5 could be assigned to C-4, C-10 and C-11 respectively. The carbons for C-4 and C-11 resonated at around δ 72-73. The signals were deshielded because those carbons were attached to the electronegative atom, oxygen. Four methyl carbons C-12, C-13, C-14 and C-15 gave a peak at δ 26.2, δ 27.8, δ 22.9 and δ 18.8. Meanwhile six secondary carbons which were C-1, C-2, C-3, C-6, C-8 and C-9 resonated at δ 43.2, 20.2, 41.9, 21.4, 22.6 and 41.1 respectively. Finally signal at δ 54.6 and δ 50.1 were attributed to the two tertiary carbons of C-5 and C-7.

From the HSQC spectrum, it was shown that C-1 coupled to H-1 and H-1’ at δ 1.05 and 1.36 respectively, C-3 coupled to H-3 and H-3’ at δ 1.27 and 1.79, C-8 coupled to H-8 and H-8’ at δ 1.08 and 1.57 and C-9 coupled to H-9 and H-9’ at δ 1.15 and 1.46 respectively.

Comparison of the spectral data with the literature values confirmed that **164** was the sesquiterpene pterodondiol.[118]

Table 3.13: ^{1}H, ^{13}C and HMBC Spectral Data of **164** in $CDCl_3$

Position	**δ_C (ppm)**	**δ_H (ppm), *J*(Hz)**	**HMBC (H→C)**
1	43.2	1.05 (1H, *m*)	5, 12
1’		1.36 (1H, *br d*) *J*=3.5	
2	20.2	1.54 (2H, *m*)	
3	44.7	1.27 (1H, *br d*) *J*=3.5	
3’		1.77 (1H, *dt*) *J*=3.5, 1.8	
4	73.1		
5	54.6	1.19 (1H, *br d*) *J*=2.7	
6	21.4	1.11 (1H, *m*)	
6’		1.92 (1H, *dd*) *J*=2.7, 5.5	
7	50.1	1.30 (1H, *d*) *J*=3.2	12
8	22.6	1.08 (1H, *m*)	
8’		1.57 (1H, *m*)	
9	41.1	1.15 (1H, *br d*) *J*=3.4	
9’		1.46 (1H, *br t*) *J*=3.4	
10	34.6		
11	72.5		
12	26.2	1.20 (3H, *s*)	4, 7, 12
13	27.8	1.20 (3H, *s*)	
14	22.9	1.11 (3H, *s*)	1, 5, 11
15	18.8	0.86 (3H, *s*)	3, 5, 9,10

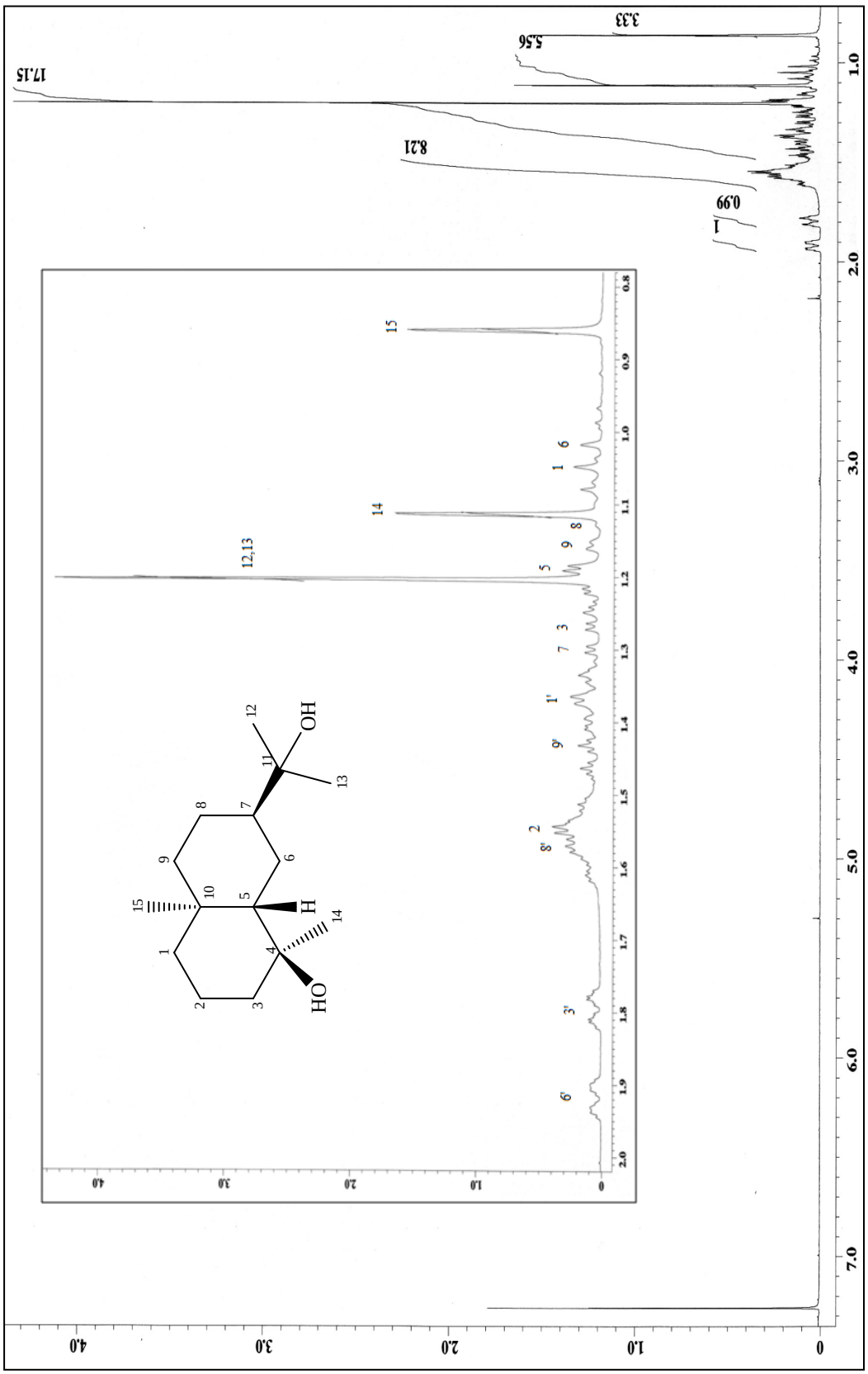


Figure 3.40: ^{1}H NMR Spectrum of **164**

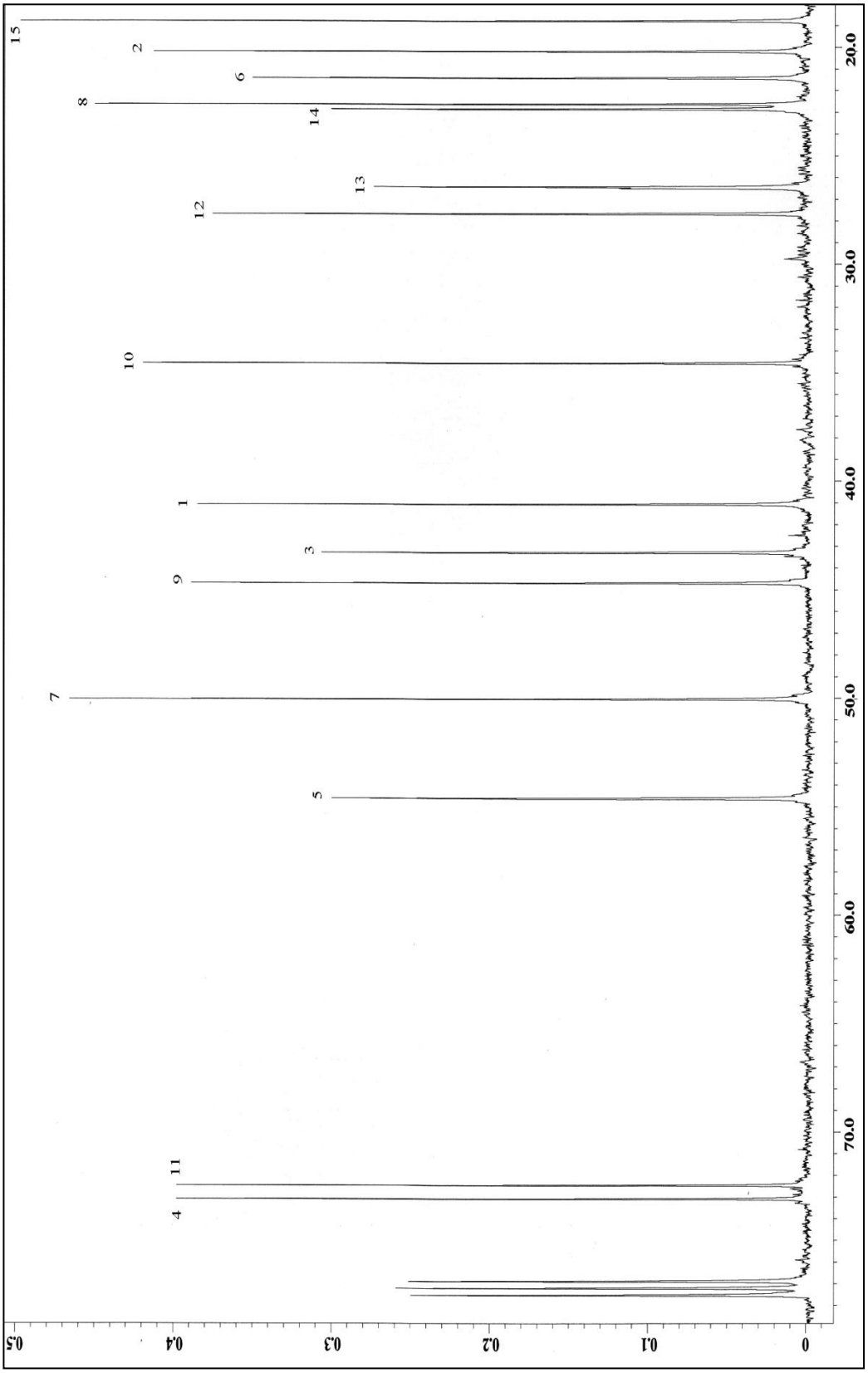
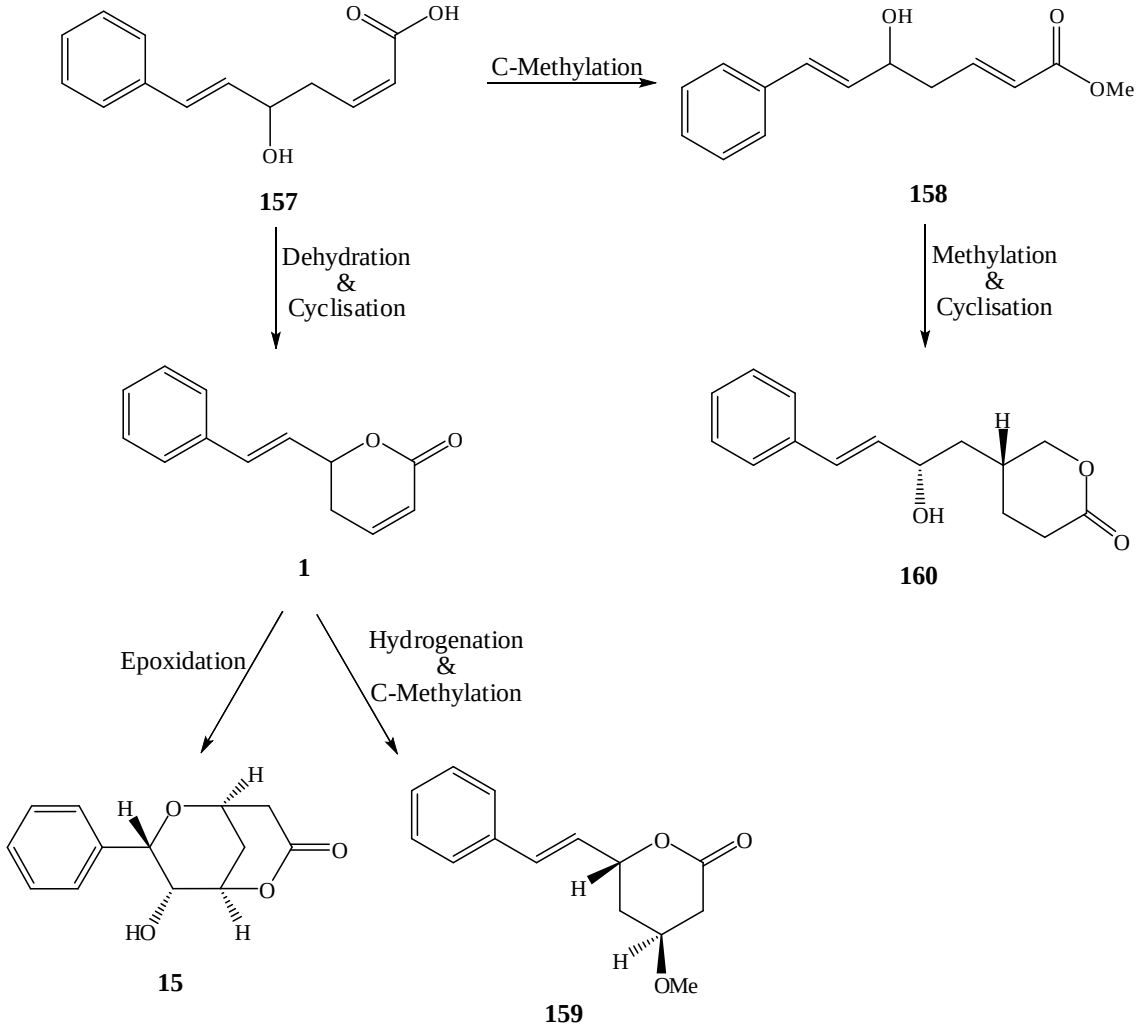


Figure 3.41: ^{13}C NMR Spectrum of **164**

3.2 Biosynthetic relationships of the Isolated Compounds

Biosynthetic relationships between all the isolated styryl-lactones are proposed in Scheme 3.2. This scheme shows that **157** is a precursor of **1** and **158**. **1** undergoes epoxidation to form **15**. It will also undergo hydrogenation and C-methylation to give **159**. **158** will undergoes methylation and cyclisation to form **160**. All proposed approaches were designed on the basis of references [119] and [120].



Scheme 3.2: Proposed biosynthetic relationship of **1**, **15**, **157**, **158**, **159** and **160**

CHAPTER 4

CYTOTOXIC ACTIVITY

4.1 Cytotoxic activity

The crude extracts of *G. tapisoides* were evaluated for cytotoxic activity against lung cancer, breast cancer and prostate cancer cell lines. The results showed that hexane and dichloromethane extracts inhibited the growth of lung cancer (A549), breast cancer (MCF-7) and prostate cancer (DU-145) cell lines. While methanol extract was not active for all three cancer cell lines. The results of bioactivity tests on the crude extracts are shown in Table 4.1-4.3.

Table 4.1: Cytotoxic activity of crude extracts from *G. tapisoides* against lung cancer cell (A549)

Extracts	Abs (490nm)	% Viability	% Death
Hexane	0.0480	2.9	97.1
DCM	0.0470	2.9	97.1
Methanol	1.4256	87.2	12.8

Positive control was 5-Fluorouracil (anti-metabolite) EC_{50}=37.2 μg/ml

Table 4.2: Cytotoxic activity of crude extracts from *G. tapisoides* against breast cancer cell (MCF-7)

Extracts	Abs (490nm)	% Viability	% Death
Hexane	0.0527	6.2	93.8
DCM	0.0543	6.4	93.6
Methanol	0.8030	94.8	5.2

Positive control was 5-Fluorouracil (anti-metabolite) EC_{50}=59.5 μg/ml

Table 4.3: Cytotoxic activity of crude extracts from *G. tapisoides* against prostate cancer cell (DU-145)

Extracts	Abs (490nm)	% Viability	% Death
Hexane	0.0623	9.4	90.6
DCM	0.0551	8.3	91.7
Methanol	0.5478	82.3	17.7

Positive control was 5-Fluorouracil (anti-metabolite) EC_{50}=6.5 μg/ml

The isolated compounds were tested on eight cancer cell lines; lung (A549), prostate (DU-145), skin (SK-MEL-5), pancreatic (BxPC-3), liver (Hep G2), colon (HT-29), breast (MCF-7) and (MDA-MB-231). Compound A exhibited cytotoxic activity against all cancer cell lines except for MDA-MB-231 (Table 4.4), while other compounds were inactive against all of the cancer cell lines.

Table 4.4: Cytotoxic activity of **1**

Cancer cell lines	**IC_{50}, μM**
A549	107.62 ± 4.67
DU-145	71.79 ± 1.61
SK-MEL-5	100.14 ± 11.84
BxPC-3	130.48 ± 7.69
Hep G2	128.73 ± 1.81
HT-29	64.17 ± 5.60
MCF-7	120.37 ± 11.11
MDA-MB-231	> 150

CHAPTER 5

CONCLUSION

5.1 CONCLUSION

The chemical study on the dichloromethane extract of the stem bark of *Goniothalamus tapisoides* has led to the isolation of eleven compounds; six styryl-lactones, a sesquiterpene, two alkaloids, benzylamide and cinnamic acid. Structural elucidations were established through spectroscopic methods; NMR (nuclear magnetic resonance), MS (mass spectrometry) and IR (infrared spectroscopy). This particular *Goniothalamus* species (*Goniothalamus tapisoides*) has not been studied chemically and biologically before.

As can be observed from Table 2.2, goniothalamin **1**, 9-deoxygoniopypyrone **15**, cinnamic acid **155** and liriodenine **40** have been previously reported from several *Goniothalamus* sp. However pterodondiol **164** and compounds **157**, **158**, **159**, **160**, **161** and **162** has not been reported so far. In addition, this is the first report of compound B as a natural product which is a possible precursor of 1 (Scheme 3.1). While **162** is a new aristolactam with *N-oxy* functional group and this is the first occurrence of such type of alkaloid in the *Goniothalamus* species.

Cytotoxic activities of the compounds were evaluated against a panel of eight cancer cell lines; lung (A549), prostate (DU-145), skin (SK-MEL-5), pancreatic (BxPC-3), liver (Hep G2), colon (HT-29), breast (MCF-7) and (MDA-MB-231). Only goniothalamin **1** showed potent activity against the cancer cell lines (Table 4.4, page 114) while the other compounds were inactive.

This study has shown that *Goniothalamus tapisoides* is a producer of many types of compounds; styryl-lactones, alkaloids, terpenes, acetogenins and it is a good source to search for potential bioactive compounds. Studies such as hemisynthesis or

microbial transformation, drug design and QSAR based on goniothalamin can be the subject of further investigations.

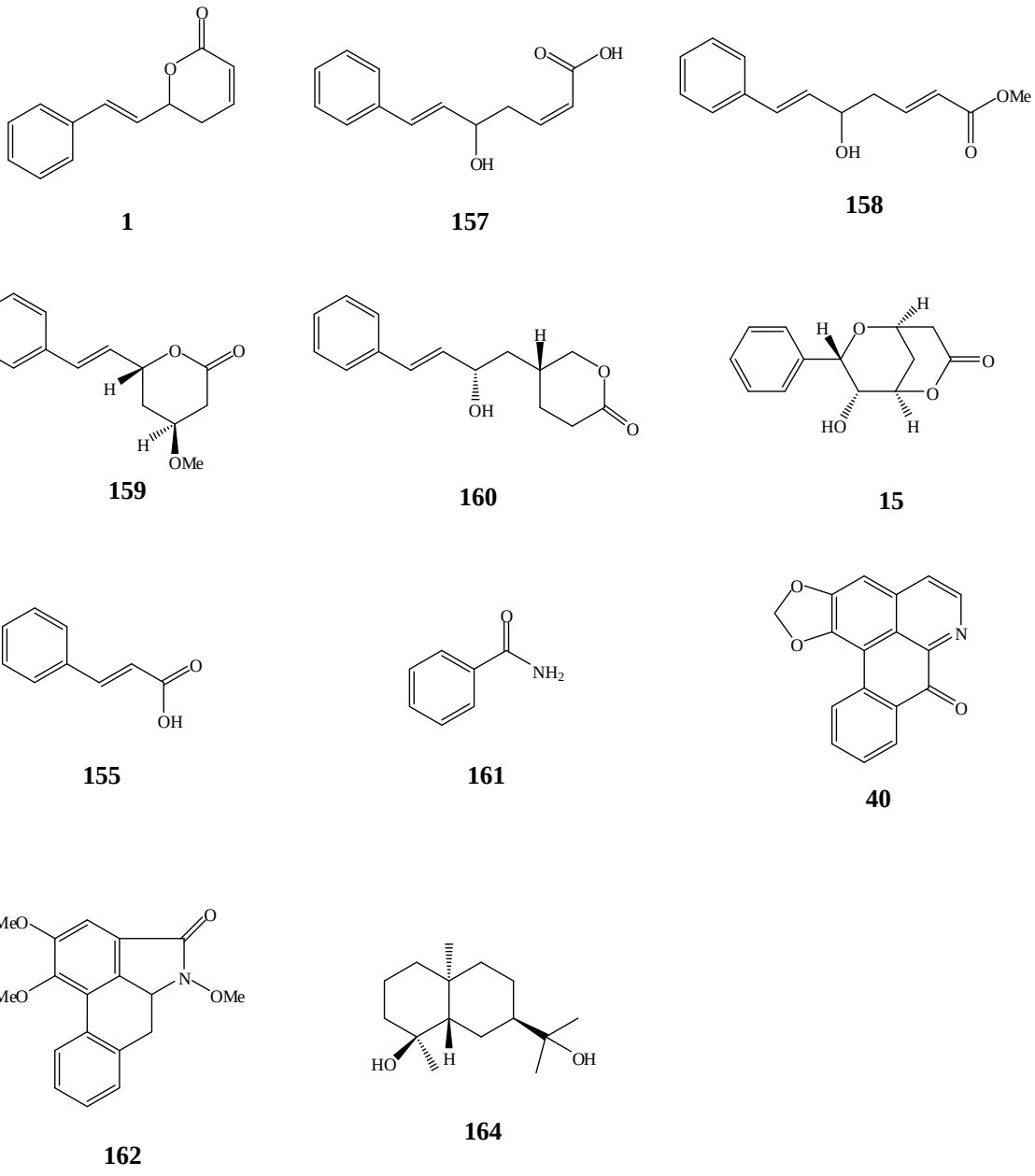


Figure 5.1: Structures of compounds isolated from *Goniothalamus tapisoides*

CHAPTER 6

EXPERIMENTAL

6.1 GENERAL METHODS

The Shimadzu 1650 PC ultraviolet-visible spectrometer were used to obtain the ultraviolet spectra, samples were dissolved in MeOH.

The infrared were taken on a Perkin Elmer Spectrum 400 FT-IR/FT-FIR Spectrometer.

NMR spectra were taken in deuterated chloroform on the JEOL JNM-LA 400 FT-NMR system. Mostly of Mostly of 13C, DEPT, HSQC and HMBC spectra were obtained from JEOL ECA 400. Chemical shifts are given in ppm on δ scale.

The mass spectra were obtained on Shimadzu GCMS-QP2010 Plus, sample were dissolved in MeOH.

Silica gel 60 (0.063-0.200 mm) and silica gel 60 (0.040-0.063 mm) were used for the column chromatography. Aluminium supported silica gel 60 F_{254} plates was used for TLC. The spots on the TLC were visualized under ultra-violet (UV) light (254 nm and 365 nm) followed with spraying by vanillin reagent.

Dragendorff's reagent

Solution A : Bismuth (III) nitrate (0.85 g) in a mixture of 10 mL glacial acid and 40 mL distilled water.

Solution B : Potassium iodide (8.0 g) in 20 mL distilled water.

Stock solution : A mixture of equal volume of solution A and solution B.

Spray reagent : The stock solution (20 mL) was diluted in a mixture of 20 mL glacial acetic acid and 60 mL distilled water.

Dragendorff's test: A positive result is indicated by the formation of orange precipitates or spots.

Vanillin reagent

Spray reagent : Diluting 10 ml of sulphuric acid with 90 mL of water in 100 mL ethanol and followed by adding 10 mg vanillin powder.

Treatment : Heat at 100°C until coloration appears.

Vanillin test : A positive result is indicated by the formation of blue or red color spots.

6.2 PLANT MATERIALS

The stem bark of *Goniothalamus tapisoides* was collected from Sarawak. Voucher specimen (HUMS 000108) is deposited in the Herbarium of Universiti Malaysia Sarawak, Kota Samarahan, Sarawak.

6.3 EXTRACTION

The dried and milled stem bark of *G. tapisoides* was first defatted with hexane for 3 days and filtered. The filtrate was concentrated under reduced pressure to yield a hexane extract. The plant material was then air dried and moistened with 25% ammonia solution and left to soak overnight. The alkaline medium ensures that any alkaloids presence in the bark will be in the free base or unionized state.

The plant material was then extracted with dichloromethane (CH_2Cl_2) by soaking them in large beakers for about three days. The dichloromethane extracted will be concentrated by using rotary evaporator. The extraction method depicted above was

continued with methanol. The yield of extracts for hexane (25 g), dichloromethane (43 g) and methanol (24 g) were obtained. The methanol crude extract was kept for future use.

6.4 ISOLATION AND PURIFICATION

20 g of dichloromethane crude was subjected to column chromatography (CC) over silica gel. The amount of silica gel used was based on the ratio of 1 g of crude extract to 30 g of silica gel. The isolation step was based on gradient elution method. The solvent systems used were hexane, hexane-dichloromethane, dichloromethane, dichloromethane-methanol and methanol to furnish 11 fractions as stated in Table 6.1.

Table 6.1: Solvents used for column chromatography of crude DCM extract

Ratio	Solvents	Fractions
100 : 0	Hexane	1
80 : 20	Hex : DCM	2
50 : 50	Hex : DCM	3
20 : 80	Hex : DCM	4
100 : 0	DCM	5
98 : 2	DCM : MeOH	6
95 : 5	DCM : MeOH	7
90 : 10	DCM : MeOH	8
80 : 20	DCM : MeOH	9
50 : 50	DCM : MeOH	10
100 : 0	MeOH	11

Each fraction was tested on thin layer chromatography (TLC) for purity. Fractions which have spots with similar R_f values and stains on the TLCs were combined and treated as a group. The combined fractions were then subjected to repeated CC or preparative TLC until a single spot on the TLC was obtained.

Table 6.2: Chromatographic Solvent Systems and Yield of Compounds from *Goniothalamus tapisoides* (stem bark)

Solvent system	Compound	Yield (mg)
50 : 50 Hexane : DCM	Goniothalamin	1300
98 : 2 DCM : MeOH	Liriodenine 9-deoxygoniopypyrone Tapisoidin Benzamide Cinnamic acid	1.2 4.4 3.2 4.8 4.0
95 : 5 DCM : MeOH	Pterodondiol	1100
90 : 10 DCM : MeOH	Goniomicin A	12.3
80 : 20 DCM : MeOH	Goniomicin B	7.9
50 : 50 DCM : MeOH	Goniomicin C Goniomicin D	14.8 18.9

6.5 CYTOTOXIC ASSAY

Cytotoxicity of the compounds were evaluated against a panel of 8 cancer cell lines; lung (A549), prostate (DU-145), skin (SK-MEL-5), pancreatic (BxPC-3), liver (Hep G2), colon (HT-29), breast (MCF-7) and (MDA-MB-231). These cancer cell lines were chosen from the National Cancer Institute (NCI) list of 60 cancer cell lines for drug screening and drug treatment conditions were done according to the NCI recommendations (Boyd, 1995).

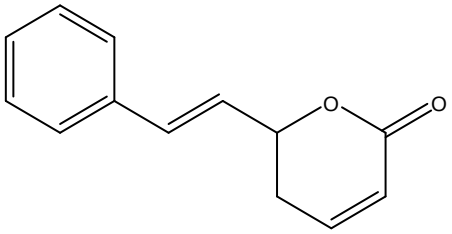
Cell lines were cultured in DMEM media supplemented with 2 mM L-glutamine, 10% foetal bovine serum, 50 µg/ml gentamycin and 2.5 µg/ml amphotericin B, maintained in a 37ºC humid atmosphere of 5% CO_2 cell incubator. Samples and drug

standards (cisplatin and vinblastine sulphate) were dissolved in DMSO and immediately diluted with DMEM media to yield a final DMSO concentration of less than 0.5% v/v.

Cells were plated into 96-well microplates at 5,000–10,000 cells per well and maintained in the cell incubator for 24 hour. Then, 100 µL of samples were introduced in triplicates to a final concentration of 15–200 µM, with the exception of sample 5 that was further diluted down to 4 µM in BxPC-3 and HT-29 cell lines. Drug standards were also introduced to a final concentration of 0.03 - 2000 µM (cisplatin) and 0.002 - 100 µM (vinblastine sulphate). Cells were further incubated for 48 hours and then, cell viability was determined according to the manufacturer protocol of a commercial MTS assay kit (CellTiter 96 AQ_{ueous}® One Solution, Promega). Culture media were carefully refreshed with 100 µL of DMEM media, followed by 20 µL per well of MTS reagent. Microplates were returned to the incubator for $1-2$ hours and absorbance of the formazan product was read on a microplate reader at 490nm with 690nm as the background wavelength (Infinite 200, Tecan, Männedorf, Swizerland). IC_{50} of samples and drug standards were determined using dose-response curves in Prism 5.02 software (GraphPad Software Inc., La Jolla, CA, USA).

6.6 General Spectral Data of Isolated Compounds

Goniothalamin



State : White crystals

Molecular formula : $C_{13}H_{12}O_2$

UV λ_{max} nm : 207, 255, 284

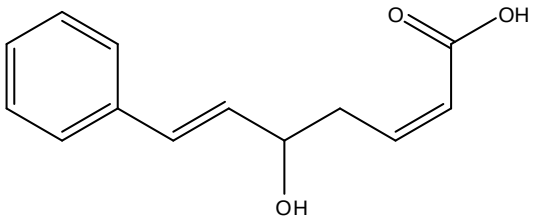
IR υ_{max} : 1722, 1249, 752

Mass Spectrum *m/z* : 200

^{1}H-NMR : Refer Table 3.2 (pg 46)

^{13}C-NMR : Refer Table 3.2 (pg 46)

Goniomicin A



State : Yellowish amorphous solid

Molecular formula : $C_{13}H_{14}O_3$

UV λ_{max} nm : 206, 251

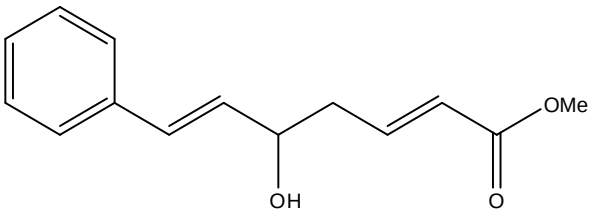
IR υ_{max} : 3344, 1668, 1634, 1329

Mass Spectrum *m/z* : 218

^{1}H-NMR : Refer Table 3.3 (pg 50)

^{13}C-NMR : Refer Table 3.3 (pg 50)

Goniomicin B



State : Yellowish amorphous solid

Molecular formula : $C_{14}H_{16}O_3$

UV λ_{max} nm : 207, 251

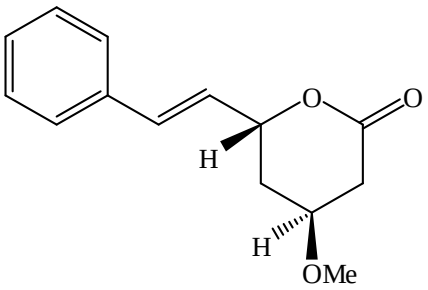
IR υ_{max} : 3436, 2926, 1718, 1659, 1209, 1168

Mass Spectrum *m/z* : 232

^{1}H-NMR : Refer Table 3.4 (pg 59)

^{13}C-NMR : Refer Table 3.4 (pg 59)

Goniomicin C



State : Yellowish amorphous solid

Molecular formula : $C_{14}H_{16}O_3$

UV λ_{max} nm : 206, 251

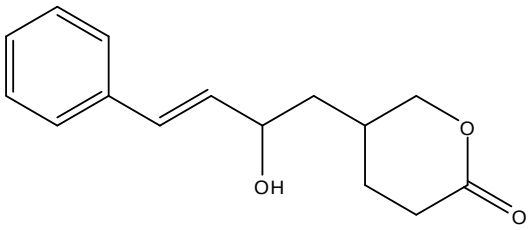
IR υ_{max} : 1731, 1241, 1090

Mass Spectrum *m/z* : 232

^{1}H-NMR : Refer Table 3.5 (pg 67)

^{13}C-NMR : Refer Table 3.5 (pg 67)

Goniomicin D



State : Pale yellow amorphous solid

Molecular formula : $C_{15}H_{18}O_3$

UV λ_{max} nm : 206, 252

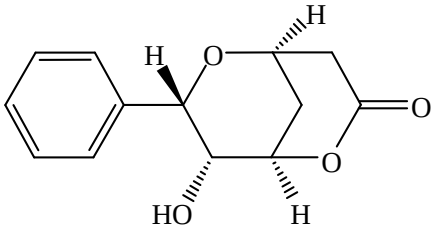
IR υ_{max} : 3413, 2917, 1662, 1204

Mass Spectrum *m/z* : 246

^{1}H-NMR : Refer Table 3.6 (pg 75)

^{13}C-NMR : Refer Table 3.6 (pg 75)

9-deoxygoniopypyrone



State : White powder

Molecular formula : $C_{13}H_{14}O_4$

UV λ_{max} nm : 206, 258

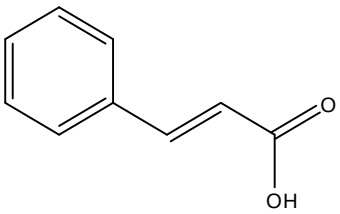
IR υ_{max} : 2932, 1720

Mass Spectrum *m/z* : 234

^{1}H-NMR : Refer Table 3.7 (pg 83)

^{13}C-NMR : Refer Table 3.7 (pg 83)

Cinnamic acid



State : White amorphous solid

Molecular formula : $C_9H_8O_2$

UV λ_{max} nm : 216, 271

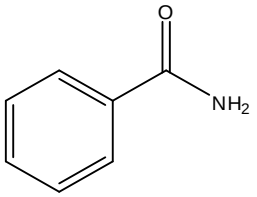
IR υ_{max} : 3362, 1658, 1601

Mass Spectrum *m/z* : 147

^{1}H-NMR : Refer Table 3.10 (pg 87)

^{13}C-NMR : Refer Table 3.10 (pg 87)

Benzylamide



State : Brown amorphous solid

Molecular formula : C_7H_7ON

UV λ_{max} nm : 207, 252

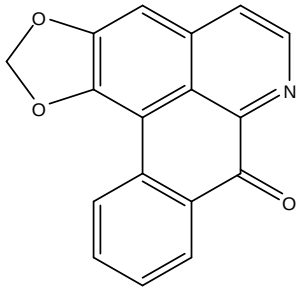
IR υ_{max} : 3384, 3189, 1647, 1617

Mass Spectrum *m/z* : 121

^{1}H-NMR : Refer Table 3.9 (pg 91)

^{13}C-NMR : Refer Table 3.9 (pg 91)

Liriodenine



State : Yellow amorphous solid

Molecular formula : $C_{17}H_9O_3N$

UV λ_{max} nm : 209, 306

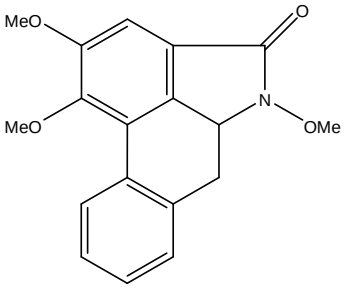
IR υ_{max} : 1728, 965, 865

Mass Spectrum *m/z* : 275

^{1}H-NMR : Refer Table 3.10 (pg 95)

^{13}C-NMR : Refer Table 3.10 (pg 95)

Tapisoidin



State : Brown amorphous solid

Molecular formula : $C_{18}H_{17}O_4N$

UV λ_{max} nm : 209, 251, 277, 322

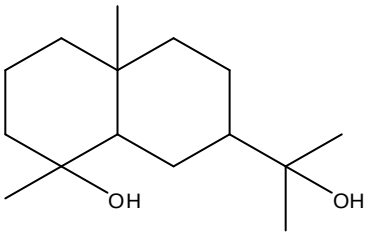
IR υ_{max} : 2938, 1715

Mass Spectrum *m/z* : 311

^{1}H-NMR : Refer Table 3.12 (pg 100)

^{13}C-NMR : Refer Table 3.12 (pg 100)

Pterodondiol



State : Colorless needles

Molecular formula : $C_{15}H_{28}O_2$

UV λ_{max} nm : 205

IR υ_{max} : 3391, 2971, 2930

Mass Spectrum *m/z* : 222

^{1}H-NMR : Refer Table 3.13 (pg 108)

^{13}C-NMR : Refer Table 3.13 (pg 108)

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