Chemical Studies and Cytotoxic Activity of *Goniothalamus undulatus* Ridl.

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Thesis Title Chemical Studies and Cytotoxic Activity of *Goniothalamus undulatus* Ridl.

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Dean of Graduate School

ชื่อวิทยานิพนธ์ การศึกษาสารเคมีและฤทธิ์ความเป็นพิษต่อเซลล์ของ *Goniothalamus undulatus* Ridl.

ผู้เขียน นางสาว สุกัญญา ตันติธนพร

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ปีการศึกษา 2551

บทคัดย่อ

Goniothalamus undulatus Ridl. เป็นต้นไม้ขนาดเล็กพบได้ในป่าทางภาคใต้ของประเทศไทย การศึกษาองค์ประกอบทางเคมีของพืชชนิดนี้ สามารถแยกสารสำคัญได้ 2 กลุ่ม คือ กลุ่ม styryl lactones 3 ชนิด ได้แก่ 5-acetoxyisogoniothalamin oxide, O-acetylaltholactone และ altholactone และกลุ่ม annonaceous acetogenins 4 ชนิด ได้แก่ annonacin, *cis*-annonacin, goniothalamicin และ *cis*-goniothalamicin ซึ่งสารดังกล่าวแยกได้จากสารสกัดในชั้น dichloromethane จากส่วนรากของพืช จากการศึกษาฤทธิ์ความเป็นพิษต่อเซลล์กับเซลล์มะเร็ง 3 ชนิด คือ เซลล์มะเร็งปอด (COR-L23) เซลล์มะเร็งเต้านม (MCF-7) และเซลล์มะเร็งตับ (HepG2) และเซลล์ปกติปอด 1 ชนิด คือ MRC-5 โดยใช้ sulphorhodamine B (SRB) assay พบว่า สารในกลุ่ม annonaceous acetogenins มีความเป็นพิษต่อเซลล์มะเร็งสูงกว่าสารในกลุ่ม styryl lactones ซึ่งสารแต่ละกลุ่มแสดงค่า IC_{50} ต่อเซลล์มะเร็งอยู่ในช่วง < 0.17-2.46 μM และ 7.37-77.58 μM ตามลำดับ อย่างไรก็ตามสารในกลุ่ม annonaceous acetogenins มีพิษต่อเซลล์ปกติสูงกว่าสารในกลุ่ม styryl lactones เช่นกัน ซึ่งสารแต่ละกลุ่มแสดงค่า IC_{50} อยู่ในช่วง 11.82-31.44 μM และ 48.67-102.82 μM ตามลำดับ

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ABSTRACT

Goniothalamus undulatus Ridl. is a small three growing in Peninsular Thailand. The investigation of chemical constituents of this plant led to the isolation of three styryl lactones including, 5-acetoxyisogoniothalamin oxide, O-acetylaltholactone and altholactone and four annonaceous acetogenins including, annonacin, *cis*-annonacin, goniothalamicin and *cis*-goniothalamicin. These compounds were isolated from dichloromethane extract of the roots and subjected to sulphorhodamine B (SRB) cytotoxicity assay against three types of cancer cell lines; large cell lung carcinoma (COR-L23), human breast adenocarcinoma (MCF-7) and human hepatocellular liver carcinoma (HepG2) and one type of normal cell line; human fetal fibroblast cell line (MRC-5). Annonaceous acetogenins showed higher cytotoxic activity against all types of cancer cell lines than styryl lactones with IC_{50} values in range of < 0.17-2.46 μM and 7.37-77.58 μM, respectively. However annonaceous acetogenins also have higher cytotoxic activity against normal cell line than styryl lactones with IC_{50} values in range of 11.82-31.44 μM and 48.67-102.82 μM, respectively.

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Sukanya Tantithanaporn

CONTENTS

CONTENTS (cont.)

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES (cont.)

LIST OF SCHEMES

LIST OF ABBREVIATIONS AND SYMBOLS

$[\alpha]_D$	specific rotation
δ	chemical shift in ppm
λ_{max}	maximum wavelength
ν_{max}	wave number
Ac	acetyl
c	concentration
COSY	correlation spectroscopy
d	doublet (for NMR signals)
EIMS	electron-impact mass spectroscopy
ESIMS	electron-sprayed ionization spectroscopy
HMBC	heteronuclear multiple bond correlation
HMQC	heteronuclear multiple-quantum coherence
IC_{50}	inhibitory concentration at 50% of tested subject
IR	infrared
J	coupling constant
m	multiplet (for NMR signals)
MS	mass spectrometry
m/z	mass-over-charge ratio
NMR	nuclear magnetic resonance
ppm	part per million
q	quartet (for NMR signals)
s	singlet (for NMR signals)
SRB	sulphorhodamine B
t	triplet (for NMR signals)
THF-ring	tetrahydrofuran ring

CHAPTER 1

INTRODUCTION

1.1 Potential of natural products as anticancer

Cancer is a group of diseases characterized by uncontrolled growth and spread of abnormal cells. If the spread is not controlled, it can result in death. In Thailand, the report of Health Information Unit, Bureau of Policy and Strategy, Department Secretary of Ministry of Public Health showed that cancer is the first most common causes of death in Thailand. Number of deaths in 2007 are 53,434. In US, about 565,650 Americans are expected to die of cancer in 2008, more than 1,500 people a day (American Cancer Society, 2008).

Cancer treatment may vary depending upon the type of cancer, the stage of cancer and the goal of treatment. Cancer treatment aims to prevent the cancer from spreading locally or recurring relapsing at sites distant from the original location (metastasis). Cancer therapy may consist of one or more treatment modalities delivered concurrently or in sequence, including surgery, radiation, chemotherapy, immunotherapy and hormone therapy.

Chemotherapy is the general term for any treatment involving the use of drugs to kill cancer cells. Anticancer chemotherapy may consist of single drugs or combinations of drugs. Chemotherapy can be administered through a vein, injected into a body cavity or delivered orally in the form of a pill. Chemotherapy is different from surgical or radiation therapy in that the cancer-fighting drugs circulate in the blood to parts of the body where the cancer may have spread and can kill or eliminate cancer cells at sites great distances from the original tumor. As a result, chemotherapy is considered as systemic treatment.

The ideal anticancer chemotherapeutic agent should be either highly toxic to cancer cells or cause such cells to revert to normal cell types. It should show little or no toxicity and have a broad spectrum of activity.

New anticancer drugs may be developed by (1) synthetic procedures using new biochemical and pharmacologic concepts and structure-activity relationships, (2) from natural

products by identifying and exploiting natural agents, (3) by screening of new synthetic compounds made for other purposes (Mereyala and Joe, 2001).

Regarding sample sources, higher plants have provided many effective, clinically useful anticancer drugs such as vinca alkaloids, taxus diterpenes, camptotheca alkaloids, podophyllum lignans, etc. (Table 1)

Table 1: Anticancer drug from plant source (Itokawa et al., 2008)

Plant source	Compound	Trademark	Use	Mechanism of action
***Vinca* species**				
- *Vinca rosea*	Vinblastine	Velban®	Treatment of Hodgkin's and non-Hodgkin's lymphoma, testicular, lung, head and neck, breast and renal carcinomas.	Binds to tubulin and inhibits microtubule formation, therefore arresting the cell at metaphase by disrupting the formation of the mitotic spindle.
	Vincristine	Oncovin®, Vincasar®	Treatment of leukemias, Hodgkin's and non-Hodgkin's lymphomas, neuroblastoma and rhabdomyosarcoma.	Binds to microtubular protein of mitotic spindle causing metaphase arrest.
***Taxus* species**				
- *Taxus brevifolia*	Paclitaxel	Onxol™, Taxol®	Treatment of advanced carcinoma of the ovary in combination with cisplatin and metastatic carcinoma of the ovary and breast.	Maintaining microtubule assembly inhibits mitosis and cell death.

Plant source	Compound	Trademark	Use	Mechanism of action
***Camptotheca* species**				
- *Camptotheca acuminata*	Camptothecin	-	Camptothecin pooly water soluble, semi-synthetic, more water-soluble analogs including topotecan (Hycamptin®) and irinotecan (Camptosar®) were developed. These two compounds are used primaly against advanced ovarian and metastatic colorectal cancers, respectively.	Binds reversibly to topoisomerase I and stabilize the cleavable complex so that relegation of the cleaved DNA strand cannot occur.
***Podophyllum* species**				
- *Podophyllum peltatum*	Podophyllotoxin	-	Podophyllotoxin is also extremely toxic, thus failed the NCI's phase I antitumor drug clinical trials in the 1970s. It was developed to	Binds to tubulin, as inhibitor of tubulin polymerization. (Saitoh et al., 2008)

Plant source	Compound	Trademark	Use	Mechanism of action
			clinically useful anticancer drugs etoposide and teniposide are used to treat small cell lung, testicular cancers and leukemia.	

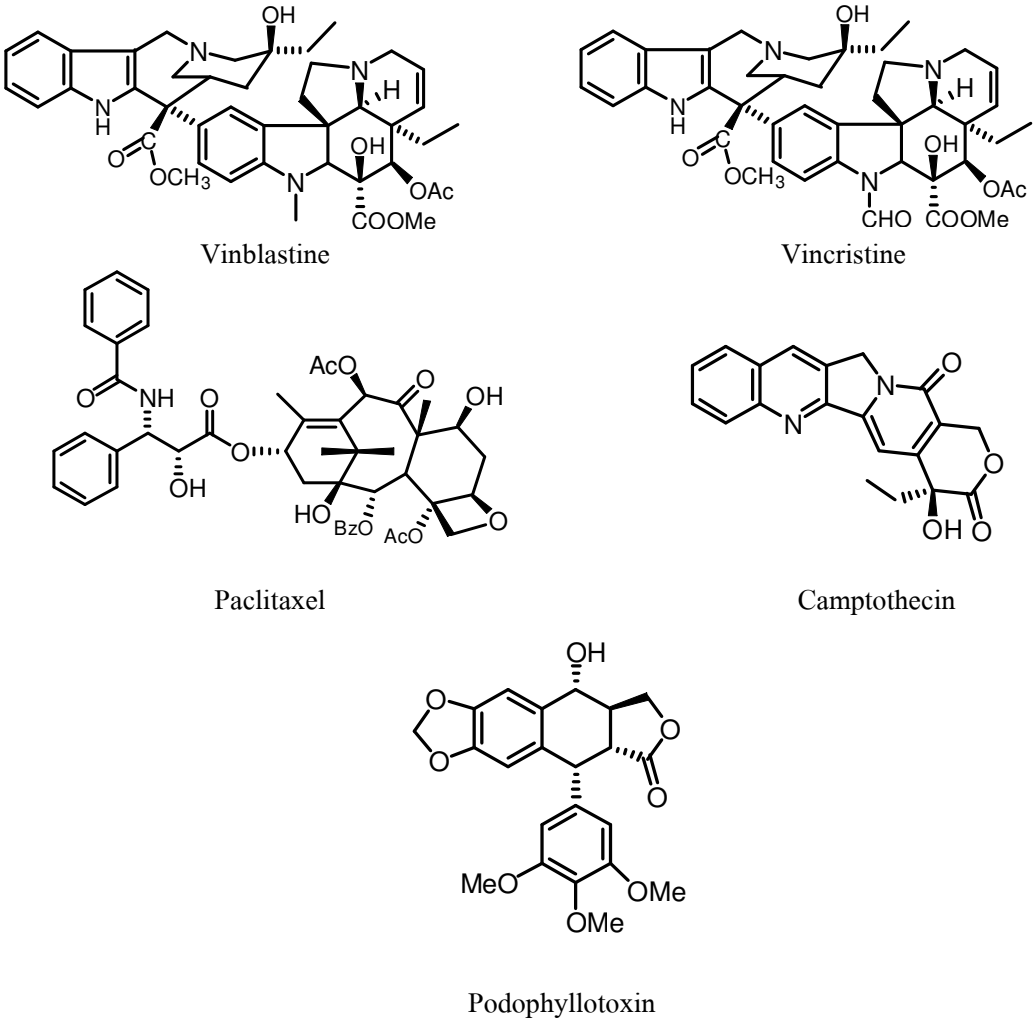


Figure 1: The structure of anticancer drugs from plant source

Many cytotoxic compounds have been isolated from plants which are excellent candidates for further development toward anticancer clinical trial. For example, neo-transhinlactone, tanshinone diterpenoid, was isolated from roots of *Salvia miltiorrhiza*. This compound showed potent and selective activity against the MCF-7 breast cancer cell line (IC_{50} = 0.6 μg/ml). Therefore, neo-tanshinlactone become attractive candidate for further anti-breast cancer drug (Wang et al., 2006).

Curcumin, was isolated from *Curcuma longa*, showed potent anti-oxidative and anti-inflammatory effects, cytotoxicity against tumor cells, and antitumor-promoting activity (Aggarwal et al., 2003). 4-Ethoxycarbonyl curcumin analogs were found to be potent anti-

androgen receptor agent and were considered to be a promising drug candidate for the treatment of prostate cancer (Lin et al., 2006)

Several quassinoids which were isolated from *Simaroubaceous* plants, showed significant cytotoxicity against three multidrug-resistant cancer cell lines, KB-7d and KB-CPT (Murakami et al., 2004). Their synthesized analogs have attracted much attention because of the wide spectrum of their biological properties. Bruceolides, which were isolated from the genus *Brucea*, showed antileukemic and antimalarial activities (Guo et al., 2005)

Therefore, plants have been a very viable source of clinically useful compounds, leads for synthetic modification and tools for mechanistic studies. The compound which shows potent and selective against cancer cells was considered to be the further anticancer drug.

1.2 *Goniothalamus* (Blume) Hook. f. & Thomson

General Description

The genus *Goniothalamus* consists of over 130 species of shrubs and small to large trees which widely distributed in lowland and submontane tropical forests in Southeast Asia (Saunders, 2002; Saunders and Chalermglin, 2008). The center of diversity lies in Sumatra, Peninsular Malaysia and Borneo (Saunders, 2003).

The genus is characterized by axillary (or slightly supra-axillary) flowers that are generally pendent (Saunders, 2003). *Goniothalamus* flowers have a whorl of three sepals and two whorls of three petals each. The inner petals are smaller than the outer and are apically connivent to form a mitriform dome that covers the reproductive organs. The flowers are bisexual with numerous free stamens and carpels. The staminal connectives are very variable in shape with truncate (*Goniothalamus miquelianus*), short apiculate (*G. ridleyi*), long apiculate (*G. wrayi*) and elongated forms (*G. longistaminus*). The pollen is released as tetrads. The fruits are apocarpous, with individual monocarps (Saunders, 2002; Saunders and Chalermglin, 2008).

In Thailand, 25 species of the genus are recognized (Saunders and Chalermglin, 2008) as follows:

1. *Goniothalamus aurantiacus* R. M. K.
2. *Goniothalamus calvicarpus* Craib
3. *Goniothalamus cheliensis* H. H. Hu
4. *Goniothalamus elegans* Ast
5. *Goniothalamus expansus* Craib
6. *Goniothalamus giganteus* (Wall. ex) Hook. f. & Thomson
7. *Goniothalamus griffithii* Hook. f. & Thomson
8. *Goniothalamus laoticus* (Finet & Gagnep.) Ban
9. *Goniothalamus latestigma* C. E. C. Fisch.
10. *Goniothalamus macrophyllus* (Blume) Hook. f. & Thomson
11. *Goniothalamus maewongensis* R. M. K. Saunders & Chalermglin
12. *Goniothalamus malayanus* Hook. f. & Thomson
13. *Goniothalamus repevensis* Pierre ex finet & Gagnep.
14. *Goniothalamus ridleyi* King
15. *Goniothalamus rongklanus* R. M. K. Saunders & Chalermglin
16. *Goniothalamus rotundisepalus* M. R. Hend.
17. *Goniothalamus sawtehii* C. E. C. Fisch.
18. *Goniothalamus scortechinii* King
19. *Goniothalamus tamirensis* Pierre ex finet & Gagnep.
20. *Goniothalamus tapis* Miq.
21. *Goniothalamus tavoyensis* Chatterjee
22. *Goniothalamus tenuifolius* King
23. *Goniothalamus tortilipetalus* M. R. Hend.
24. *Goniothalamus undulatus* Ridl.
25. *Goniothalamus uvarioides* King


Goniothalamus aurantiacus R. M. K.


Goniothalamus giganteus (Wall. ex) Hook. f. & Thomson


Goniothalamus laoticus (Finet & Gagnep.) Ban

Goniothalamus macrophyllus (Blume) Hook. f. & Thomson

Goniothalamus repevensis Pierre ex finet & Gagnep.

Goniothalamus sawtehii C. E. C. Fisch.


Goniothalamus scortechinii King


Goniothalamus tapis Miq.

Figure 2: Pictures of flowers and fruits of some species of *Goniothalamus* reported in Thailand (Saunders and Chalermglin, 2008)

Traditional medicinal use

Several of *Goniothalamus* species have been used as traditional medicine. In Thailand, the essence derived from boiling of *G. macrophyllus* is used to nourish the blood and invigorate the body (Thonghom, 1993). In Malaysia a decoction of roots of this plant is used to aid abortion or to aid recovery from childbirth and a decoction of the leaves is used for steaming the body in case of fever (Wiart, 2000), whereas Indonesians drink a decoction of the roots for

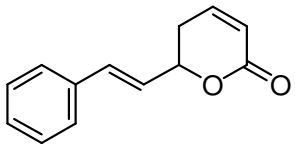
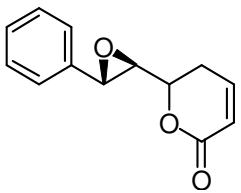
the same purpose (Wiart, 2006). The roots of *G. tapis* are used as abortifacient (Wiart, 2000). In Taiwan, extracts of the seeds of *G. amuyon* have been used for the treatment of edema and rheumatism (Wu et al., 1991). The stem bark of G. *dolichocarpus* is burnt to repel insects, especially mosquitoes (Goh et al., 1995).

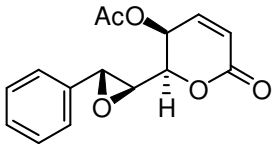
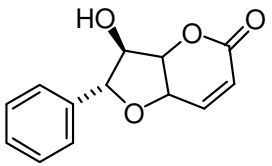
Three main classes of compounds were found in *Goniothalamus spp*., including styryl lactones, annonaceous acetogenins and alkaloids (Tian et al., 2006).

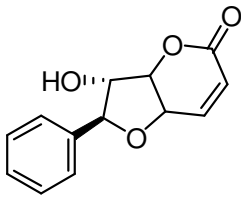
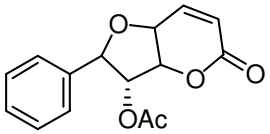
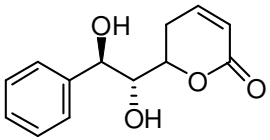
1. Styryl lactones

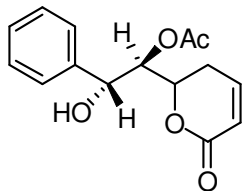
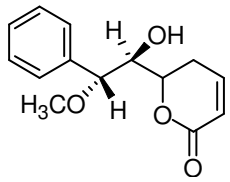
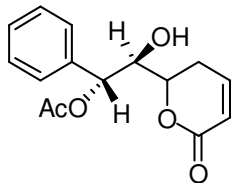
Styryl lactones are low molecular weight phenolic compounds which have been reported as constituents of Lauraceae, Piperaceae and Annonaceae (Leboeuf et al., 1982). Plants of the genus *Goniothalamus* are a rich source of styryl lactones (Table 2). Testing of these compounds for cytotoxicity showed that styryl lactones are toxic for several human tumors cell lines.

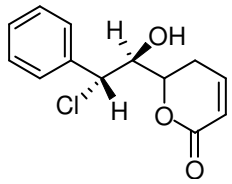
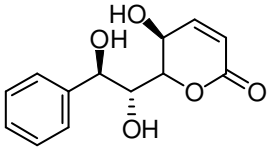
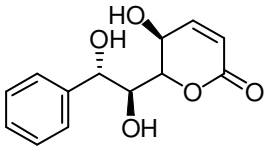
Table 2: Stylryl lactone from *Goniothalamus spp*.

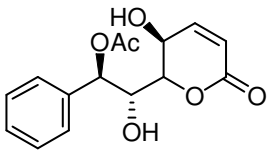
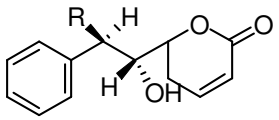
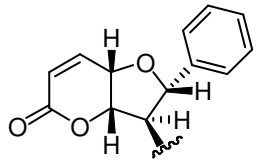
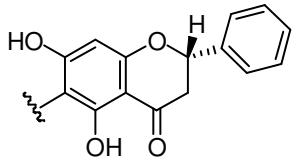
Compound	Plant source	(part of plant)	Activity	Reference
Goniothalamin				
	*G. amuyon*	(stem and leaf)	- Cytotoxic activity (IC_{50})	El-Zayat et al.,1985;
	G. borneensis	(stem bark)	P388 = 0.75 ; WEHI164 = 1.70;	Sam et al., 1987;
	G. cardiopetalus	(stem bark)	MOLT4 < 1; HepG2 = 0.31; Hep3B =	Ahmad et al., 1991;
	G. dolichocarpus	(stem bark)	1.07; MDA-MB-231 = 1.07 and MCF-7	Goh et al., 1995;
	G. giganteus	(stem bark)	= 4.64 µg/ml	Cao et al., 1998;
	G. grifithii	(stem branch)	LoVo = 46; 3AO = 14.6; HL-60 = 4.4	Hisham et al., 2003;
	G. macrophyllus	(root)	and U937 = 9 µM	Lan et al., 2003;
	G. uvaroides	(root)	- High toxicity againt the lavea of *Aedes*	Mu et al., 2003;
			aegypti LC_{50} = 15 µg/ml	Lan et al., 2005;
				Tian et al., 2006
Goniothalamin epoxide				
	*G. amuyon*	(stem and leave)	- Cytotoxic activity (IC_{50})	Sam et al., 1987;
	G. dolichocarpus	(stem bark)	NUGC = 32.1; HONE-1 = 36.3; HepG2	Goh et al., 1995;
	G. macrophyllus	(root)	= 0.19; HepG3B = 3.29; MDA-MB-231	Lan et al., 2003;
			= 1.23 and MCF-7 = 1.94 µg/ml	Lan et al., 2005

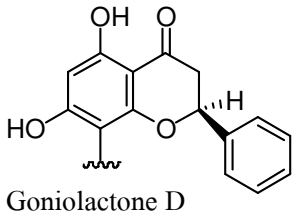
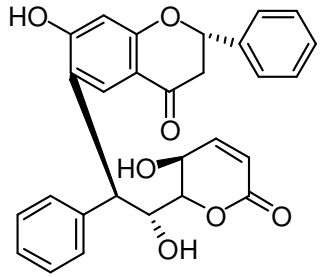
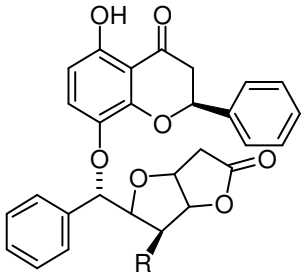
Compound	Plant source	(part of plant)	Activity	Reference
5- Acetoxyisogoniothalamin oxide 	*G. sesquipeedalis* *G. arvensis*	(stem bark) (stem bark)	- NADH oxidase activity of mammalian respiratory chain IC_{50} = 3.0 µM and IC_{100} = 22 µM	Hasan et al., 1994; Paris et al., 2000
Altholactone (Goniothalenol) 	*G. arvensis* *G. borneensis* *G. cardiopetalus* *G. giganteus* *G. grifithii* *G. malayanus*	(stem bark) (stem bark) (stem bark) (stem bark) (stem bark) (stem bark)	- Cytotoxic activity (IC_{50}) P388 > 30; WEHI 164 > 30 and THP-1 > 30 µg/ml HepG2 = 7.07 and HepG2-R = 6.17 µM - NADH oxidase activity of mammalian respiratory chain IC_{50} = 25 µM - Induced apoptosis on human HL-60 promyelocytic leukemia cells	El-Zayat et al.,1985; Cao et al., 1998; Hisham et al., 2000; Paris et al., 2000; Inayat-Hussain et al., 2002; Tian et al., 2006

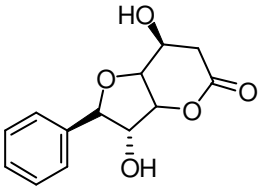
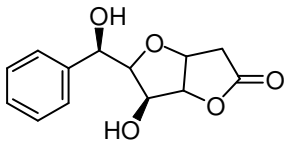
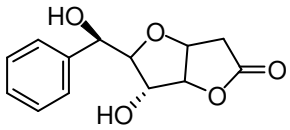
Compound	Plant source	(part of plant)	Activity	Reference
Isoaltholactone 	*G. malayanus* *G. montanus* *G. tapis*	(stem bark) (stem bark and leaf) (root)	- Mildly cytotoxic against the cell lines tested	Colegate et al., 1990
O- Acetylaltholactone 	*G. arvensis*	(stem bark)	- NADH oxidase activity of mammalian respiratory chain IC_{50} = 4.7 µM and IC_{100} = 32 µM	Paris et al., 2000
Goniodiol 	*G. amuyon* *G. cardiopetalus* *G.dolichocarpus* *G. giganteus* *G. grifithii*	(stem) (stem bark) (stem bark) (stem bark) (stem branch)	- Cytotoxic activity (IC_{50}) A-549 = 0.12; MCF-7 = 8.27; HT-29 = 2.45; HepG2 = 9.15; Hep3B = 17.21 and MDA-MB-231 = 8.80 µg/ml	Fang et al., 1991a; Goh et al., 1995; Hisham et al., 2003; Lan et al., 2005; Tian et al., 2006

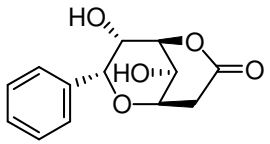
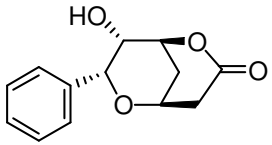
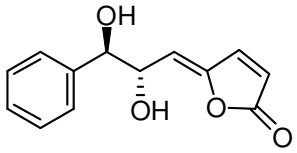
Compound	Plant source	(part of plant)	Activity	Reference
Goniodiol-7- monoacetate 	*G. amuyon* *G. grifithii* *G. sesquipedalis*	(leaf) (bark) (leaf and twig)	- Cytotoxic activity (IC_{50}) KB < 0.1; P388 < 0.1; RPMI < 0.1; TE671 < 0.1; NUGC = 4.12; HONE-1 = 5.69; Hep3B = 7.85; HepG2 = 4.63 and MDA-MB-231 = 8.05 µg/ml	Wu et al., 1991; Lan et al., 2003; Lan et al., 2005
8- Methoxygoniodiol 	*G. amuyon*	(stem and leaf)	- Cytotoxic activity (IC_{50}) NUGC = 167.6; HONE-1 = 239.7; HepG2 = 4.63; Hep3B = 6.15 µg/ml	Lan et al., 2003; Lan et al., 2005
Goniodiol-8-monoacetate 	*G. amuyon*	(leaf)	- Cytotoxic activity (IC_{50}) HepG2 = 4.63; MDA-MB-231 = 8.05 µg/ml	Lan et al., 2005

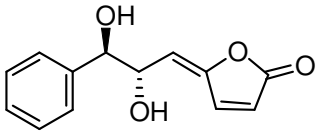
Compound	Plant source	(part of plant)	Activity	Reference
8- Chlorogoniodiol				
	*G. amuyon*	(stem and leaf)	- Cytotoxic activity (IC_{50}) NUGC = 31.0; HONE-1 = 14.87; HepG2 = 0.64; Hep3B = 3.64, MDA-MB-231 = 1.47; MCF = 72.32 µg/ml	Lan et al., 2003; Lan et al., 2005
(5S, 6R, 7R, 8R)- Goniotriol				
	*G. amuyon* *G. borneensis* *G. giganteus*	(stem and leaf) (bark) (bark)	- Cytotoxic activity (IC_{50}) P388 = 9.2; WEHI164 = 164; THP-1 = 30; MOLT4 = 8.1; A-549 > 10; MCF = 5.9; HT-29 = 10 µg/ml	Alkofahi et al., 1989; Cao et al., 1998; Lan et al., 2003
(5S, 6R, 7S, 8S)- Goniotriol				
	*G. amuyon*	(leaf)	-	Lan et al., 2003

Compound	Plant source	(part of plant)	Activity	Reference
8-O- Acetylgoniotriol				
	*G.grifithii*	(stem)	- Cytotoxic activity (IC_{50}) Bel7402 = 55; LoVo = 43; MCF-7 = 21; 3AO = 149; HL-60 = 68; U937 = 12 µM	Mu et al., 2003
Goniolactone A-F				
	*G. cheliensis*	(root)	- Goniolactone B exhibited significant inhibitory activities toward A2780, HCT-8 and KB cells with IC_{50} values of 7.40, 4.43 and 7.23 µM respectively	Wang et al., 2002
Goniolactone A; R=			- Goniolactone A, D and F showed no significant inhibitory actives toward the tested cell line.	
			- Goniolactone C and E were not evaluated biologically due to insufficient supplies of these compounds	
Goniolactone B; R=				
				

Compound	Plant source (part of plant)	Activity	Reference
Goniolactone C; R= 			
Goniolactone D 			
Goniolactone E; R= H			
Goniolactone F; R= $COCH_3$ 			

Compound	Plant source	(part of plant)	Activity	Reference
Goniofupyrone 	*G. cardiopetalus* *G. giganteus*	(stem bark) (bark)	- Cytotoxic activity (IC_{50}) A-549 = 56.36; MCF-7 > 100; HT-29 = 38.02 µg/ml	Fang et al., 1991b; Hisham et al., 2003
Goniofufurone 	*G. arvensis* *G. borneensis* *G. cardiopetalus* *G. giganteus*	(stem bark) (bark) (stem bark) (stem bark)	- Cytotoxic activity (IC_{50}) A-549 = 4.76 µg/ml	Fang et al., 1991a; Cao et al., 1998; Hisham et al., 2003
7-epi-goniofufurone 	*G. giganteus*	(stem bark)	- Cytotoxic activity (IC_{50}) A-549 = 85.49; MCF-7 = 49.11; HT-29 > 100 µg/ml - BS LC_{50} = 475 µg/ml	Fang et al., 1991a

Compound	Plant source	(part of plant)	Activity	Reference
Goniopypyrone				
	*G. cardiopetalus*	(stem bark)	-	Fang et al., 1991a;
	G. giganteus	(stem bark)		Hisham et al., 2003
9-Deoxygoniopypyrone				
	*G. amuyon*	(stem and leave)	- Cytotoxic activity (IC_{50})	Lan et al., 2003;
	G. giganteus	(stem bark)	A-549 = 27.20; MCF-7 = 25.35; HT-29 = 7.38 µg/ml	Lan et al., 2005; Fang et al., 1991a
			- BS LC_{50} > 500 µg/ml	
Goniobutenolide A				
	*G. amuyon*	(leaf)	- Cytotoxic activity (IC_{50})	Fang et al., 1991b;
	G. borneensis	(bark)	A-549 = 3.73; MCF-7 = 7.76; HT-29 = 3.41; P388 = 6.0; WEHI164 = 22; THP-1 = 2.45; MOLT4 = 5.1; HepG2 = 5.83; Hep3B = 15.33; MDA-MB-231 = 1.36 µg/ml	Cao et al., 1998; Lan et al., 2005
	G. giganteus	(bark)		

Compound	Plant source	(part of plant)	Activity	Reference
Goniobutenolide B				
	*G. amuyon* *G. giganteus*	(leaf) (bark)	- Cytotoxic activity (IC_{50}) A-549 = 0.91; MCF-7 = 19.85; HT-29 = 2.67; HepG2 = 6.68; Hep3B = 10.99; MDA-MB-231 = 1.40 µg/ml	Fang et al., 1991b; Lan et al., 2005

2. Annonaceous acetogenins

Acetogenins have so far only been characterized from members of family Annonaceae including the genus *Goniothalamus* (Table 3).

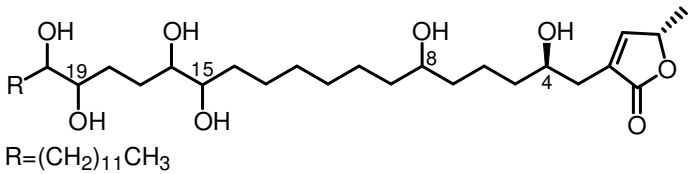
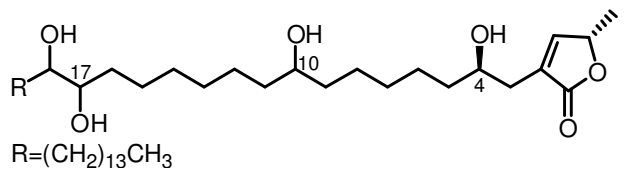
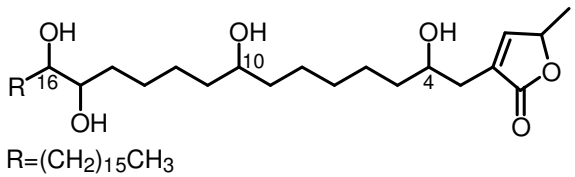
In general, acetogenins consist of a C35 or C37 carbon chain, which is presumably derived from C32 or C34 fatty acids combined with a 2-propanol unit. They are usually characterized by a long aliphatic chain bearing a terminal methyl-substituted α,β-unsaturated γ-lactone ring with one, two, three tetrahydrofuran (THF) rings, tetrahydropyran (THP) rings, epoxy rings or double bonds at the central core. The tetrahydrofuran ring can be *cis*- or *trans*-. In the case of THF rings were represented more than one in the structure, the ring can be adjacent or nonadjacent to each other.

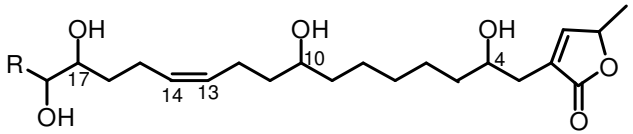
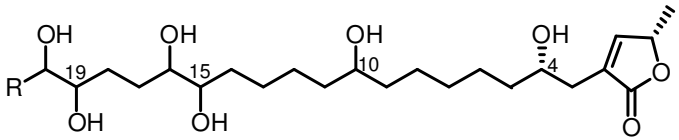
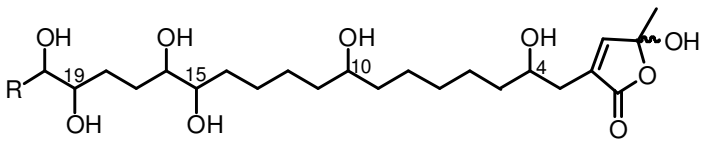
Several annonaceous acetogenins showed potent toxicity in the brine shrimp test and cytotoxic activity which have been related with a specific inhibition on the enzyme NADH-ubiquinone oxidoreductase (complex I) of mitochondria respiratory chain (Orru et al., 2003 and Ndob et al., 2009).

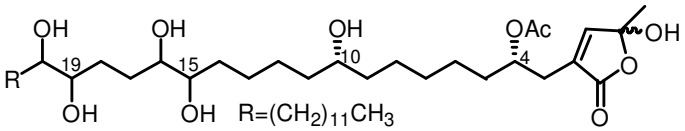
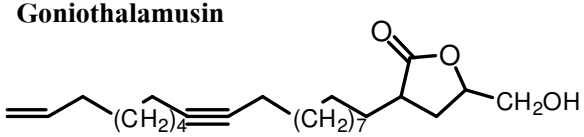
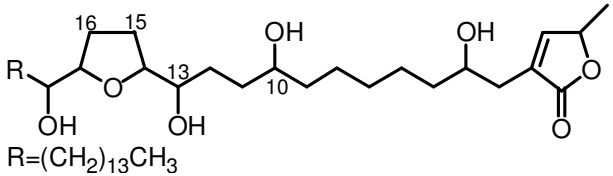
3. Alkaloids

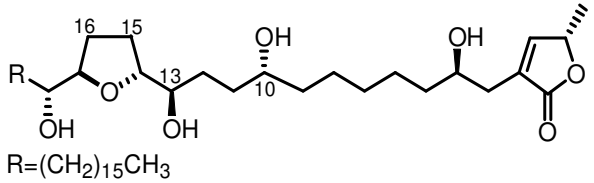
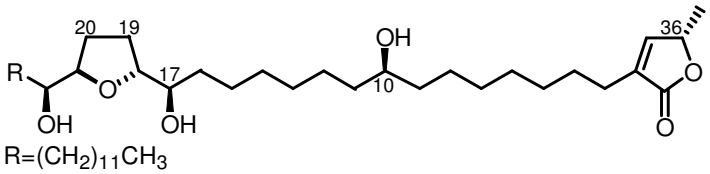
Several phenanthene lactams and aza-anthraquinone alkaloids were isolated from *Goniothalamus* spp. as cytotoxic and antimalarial compounds (Table 4).

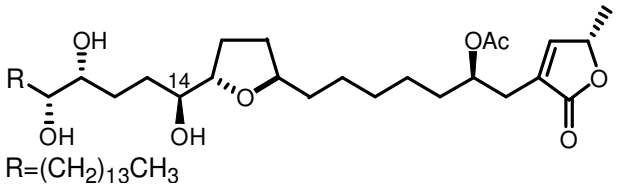
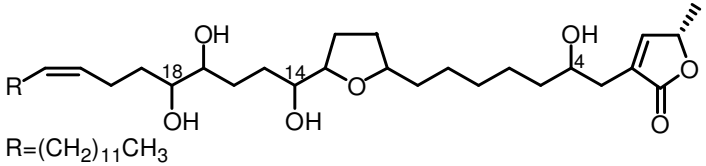
Table 3: Annonaceous acetogenins from *Goniothalamus spp*.

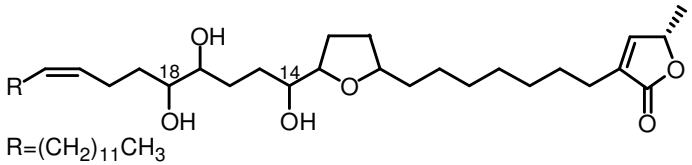
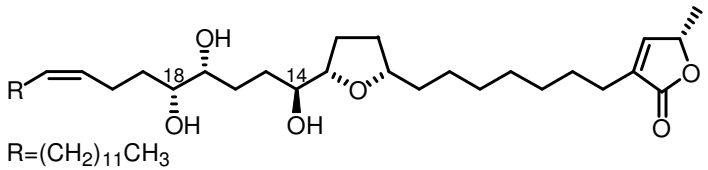
Compound	Plant source	(part of plant)	Activity	Reference
Non-THF				
Gardneri A				
	*G. gardneri*	(root)	- Cytotoxic activity (IC_{50}) KB > 10; HCT-8 > 10 and Bel 7402 = 3.6 µg/ml	Chen et al., 1998a
Gardneri B				
	*G. gardneri*	(root)	- Cytotoxic activity (IC_{50}) KB = 5.5; HCT-8 = 4.2 and Bel 7402 = 8.5 µg/ml	Chen et al., 1998a
Donbutocin				
	*G. donnaiensis*	(root)	- Cytotoxic activity (IC_{50}) HCT-8 = 4.8; Bel 7402 = 5.7 and L1210 = 0.81 µg/ml	Jiang et al., 1998a

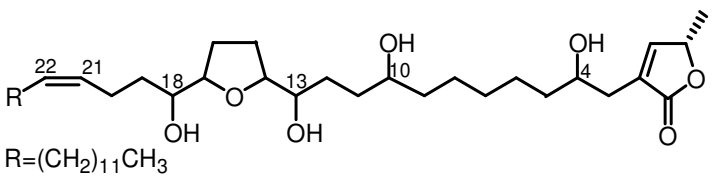
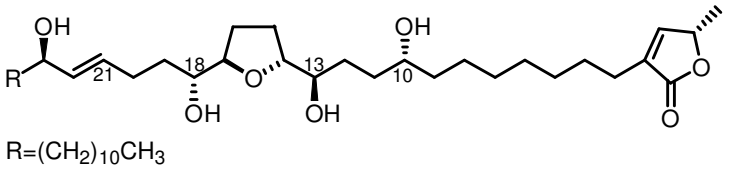
Compound	Plant source	(part of plant)	Activity	Reference
Giganin  R=$(CH_2)_{13}CH_3$	*G. giganteus*	(bark)	-	Fang et al., 1993
Donhexocin  R=$(CH_2)_{11}CH_3$	*G. donnaiensis*	(root)	- Cytotoxic activity (IC_{50}) HCT-8 = 0.82; Bel 7402 > 10 and L1210 > 10 µg/ml	Jiang et al., 1998a
Donhepocin and 34-*epi*-donhepocin  R=$(CH_2)_{11}CH_3$	*G. donnaiensis*	(root)	- Cytotoxic activity (IC_{50}) HCT-8 > 10; Bel 7402 > 10 and L1210 > 10 µg/ml	Jiang et al., 1998a

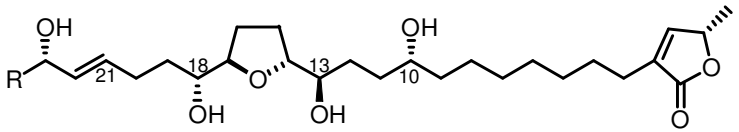
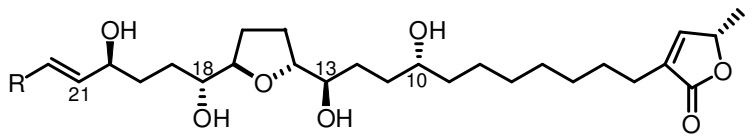
Compound	Plant source	(part of plant)	Activity	Reference
Donnaienin D and 34-*epi*-donnaienin D				
	*G. donnaiensis*	(root)	- Cytotoxic activity (IC_{50}) KB > 10; HCT-8 > 10 and Bel 7402 > 10 µg/ml	Jiang et al., 1998b
Goniothalamusin				
	*G. gardneri*	(aerial part)	-	Seidel et al., 1999
Mono- THF				
Goniothalamicin				
	*G. giganteus*	(stem bark)	- Cytotoxic activity (IC_{50}) HCT-8 = 4.8; Bel 7402 = 5.7 and L1210 = 0.81 µg/ml	Rupprecht et al., 1990

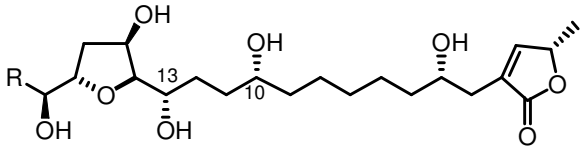
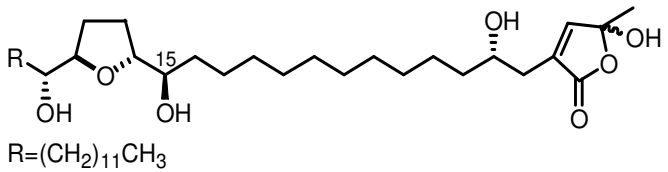
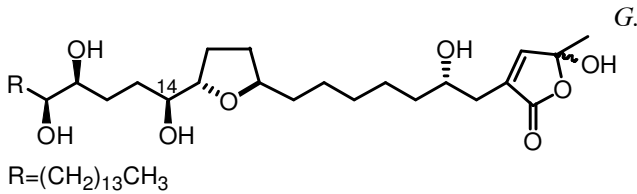
Compound	Plant source	(part of plant)	Activity	Reference
Goniotetracin 	*G. giganteus*	(bark)	- Cytotoxic activity (IC_{50}) A-549 = 3.9 x 10^{-1}; MCF-7 = 1.7; HT-29 = 1.5; A-498 = 1.5; PC-3 = 2.1 x 10^{-1} and PACA-2 = 2.6 x 10^{-2} µg/ml - BST LC_{50} < 2.0 x 10^{-1} µg/ml	Alali et al., 1998a
4-Deoxyannomontacin 	*G. giganteus*	(bark)	- Cytotoxic activity (IC_{50}) A-549 = 6.45 x 10^{-7}; MCF-7 = 5.77 x 10^{-7}; HT-29 = 1.41 x 10^{-1}; A-498 = 1.5 x 10^{-1}; PC-3 = 1.73 x 10^{-1} and PACA-2 = 1.00 x 10^{-5} µg/ml - BST LC_{50} = 0.13 µg/ml	Alali et al., 1997a

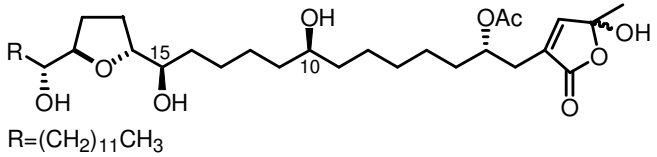
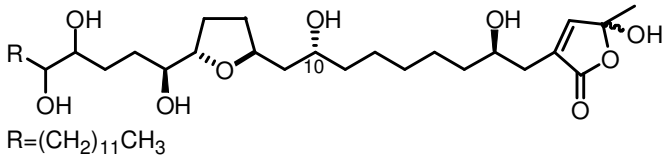
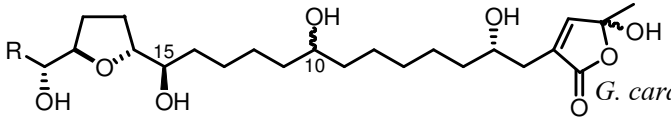
Compound	Plant source	(part of plant)	Activity	Reference
4-Acetyl gigantetrocin A 	*G. giganteus*	(bark)	- Cytotoxic activity (IC_{50}) A-549 < 10^{-2}; MCF-7 = 8.50 x 10^{-1}; HT-29 < 10^{-2}; A-498 = 1.55 x 10^{-1}; PC-3 = 1.02 and PACA-2 < 10^{-2} µg/ml - BST LC_{50} = 6.78 µg/ml	Zeng et al., 1996a
Gigantetronenin 	*G. giganteus*	(bark)	- Cytotoxic activity (IC_{50}) A-549 = 4.71 x 10^{-3}; MCF-7 = 6.03 x 10^{-1} and HT-29 = 5.37 x 10^{-2} µg/ml - BST LC_{50} = 9.4 µg/ml	Fang et al., 1992 Gu et al., 1994a

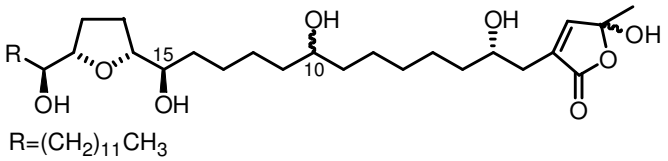
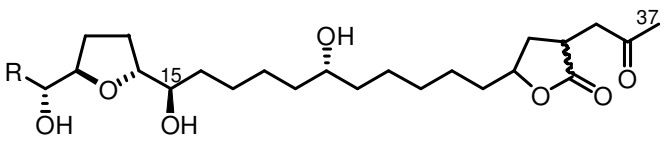
Compound	Plant source	(part of plant)	Activity	Reference
Gigantrionenin				
	*G. giganteus*	(bark)	- Cytotoxic activity (IC_{50}) A-549 = 3.94 x 10^{-3}; MCF-7 = 8.06 and HT-29 = 2.92 x 10^{-3} µg/ml - BST LC_{50} = 13.9 µg/ml	Fang et al., 1992
***cis*-Gigantrionenin**				
	*G. giganteus*	(bark)	- Cytotoxic activity (IC_{50}) A-549 = 5.99 x 10^{-2}; MCF-7 = 2.68 x 10^{-1}; HT-29 = 6.94 x 10^{-6}; A-498 = 1.39 x 10^{-2}; PC-3 = 1.11 x 10^{-1} and PACA-2 = 1.15 x 10^{-1} µg/ml - BST LC_{50} = 2.52 µg/ml	Zeng et al., 1996a

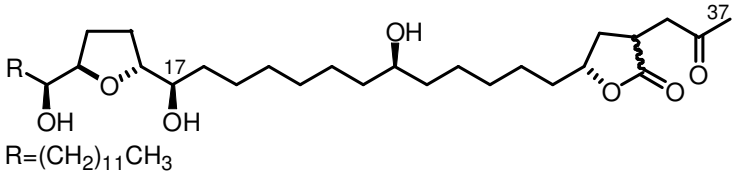
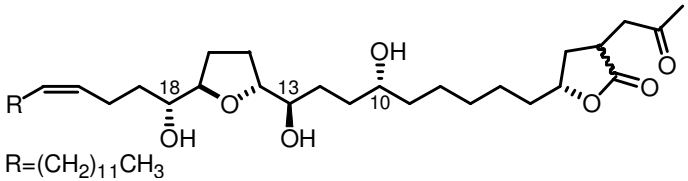
Compound	Plant source (part of plant)	Activity	Reference
Gonionenin  $R=(CH_2)_{11}CH_3$	*G. giganteus* (bark)	- Cytotoxic activity (IC_{50}) A-549 = 1.34 x 10^{-3}; MCF-7 = 4.54 x 10^{-3} and HT-29 = 1.12 x 10^{-4} µg/ml - BST LC_{50} = 21.7 µg/ml	Fang et al., 1992
Gigantransenin A  $R=(CH_2)_{10}CH_3$	*G. giganteus* (bark)	- Cytotoxic activity (IC_{50}) A-549 = 0.16; MCF-7 = 1x10^{-2}; HT-29 = 1.5; A-498 = 1.5; PC-3 = 0.18 and PACA-2 = 0.17 µg/ml - BST LC_{50} = 3.6 µg/ml	Zeng et al., 1996b

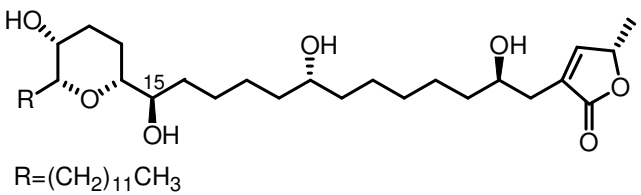
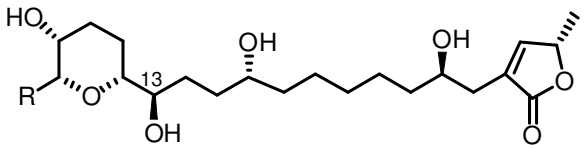
Compound	Plant source	(part of plant)	Activity	Reference
Gigantransenin B				
 $R=(CH_2)_{10}CH_3$	*G. giganteus*	(bark)	- Cytotoxic activity (IC_{50}) A-549 = 0.21; MCF-7 = 2.1 x 10^{-2}; HT-29 = 1.4; A-498 = 1.6; PC-3 = 0.71 and PACA-2 = 1.5 µg/ml - BST LC_{50} = 5.8 µg/ml	Zeng et al., 1996b
Gigantransenin C				
 $R=(CH_2)_{10}CH_3$	*G. giganteus*	(bark)	- Cytotoxic activity (IC_{50}) A-549 = 0.18; MCF-7 = 2.2 x 10^{-2}; HT-29 = 1.3; A-498 = 1.5; PC-3 = 1.5 and PACA-2 = 1.1 µg/ml - BST LC_{50} = 4.2 µg/ml	Zeng et al., 1996b

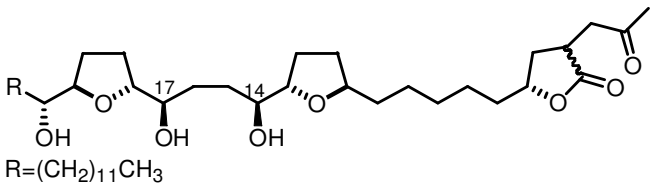
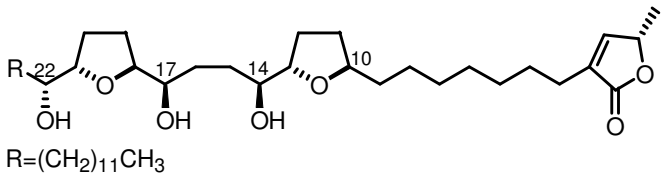
Compound	Plant source	(part of plant)	Activity	Reference
Donnaienin				
	*G. donaiesis*	(root)	- Cytotoxic activity (IC_{50}) KB > 10; HCT-8 > 10 and Bel human tumor cell = 6.7 µg/ml	Jiang et al., 1997
Donnaienin A and 34-*epi*-donnaienin A				
	*G. donnaiensis*	(root)	- Cytotoxic activity (IC_{50}) KB < 1; HCT-8 < 10 µg/ml	Jiang and Yu, 1997
Donnaienin B and 34-*epi*-donnaienin B				
	*G. donnaiesis*	(root)	- Cytotoxic activity (% inhibition) KB = 53.8 % at 10 µg/ml	Jiang and Yu, 1997

Compound	Plant source	(part of plant)	Activity	Reference
Donnaienin C and 34-*epi*-donnaienin C				
	*G. donnaiesis*	(root)	- Cytotoxic activity (IC_{50}) KB > 10; HCT-8 > 10 and Bel human tumor cell = 7.1 µg/ml	Jiang et al., 1998b
Gardnerinin and 34-*epi*-gardnerinin				
	*G. giganteus*	(bark)	- Cytotoxic activity (IC_{50}) KB > 10; HCT-8 = 6.6 and Bel 7402 = 10 µg/ml	Chen et al., 1998b
Goniodonin and 34-*epi*-goniodonin				
	*G. cardiopetalus*	(stem bark)	- Cytotoxic activity (IC_{50}) HCT-8 < 10 µg/ml	Jiang et al., 1997; Hisham et al., 2003
	G. donnaiensis	(root)		

Compound	Plant source	(part of plant)	Activity	Reference
***cis*-Goniodonin and 34-*epi*-*cis*-goniodonin**				
 R=$(CH_2)_{11}CH_3$	*G. donnaiensis*	(root)	- Cytotoxic activity (IC_{50}) HCT-8 < 10 µg/ml	Jiang et al., 1997
(2,4-*cis* and *trans*)-Xylomaticinones				
 R=$(CH_2)_{13}CH_3$	*G. giganteus*	(bark)	- Cytotoxic activity (IC_{50}) A-549 = 1.4 x 10^{-2}; MCF-7 = 7.6 x 10^{-4}; HT-29 = 7.4 x 10^{-4}; A-498 = 1.2 x 10^{-1}; PC-3 = 5.7 x 10^{-2} and PACA-2 = 7.4 x 10^{-2} µg/ml - BST LC_{50} = 0.4 µg/ml	Alali et al., 1999

Compound	Plant source	(part of plant)	Activity	Reference
(2,4 *cis* and *trans*)-Annomontacinone				
	*G. giganteus*	(bark)	- Cytotoxic activity (IC_{50}) A-549 = 1.00; MCF-7 = 2.60; HT-29 = 3.21; A-498 = 2.55 x 10^{-1}; PC-3 = 1.44 and PACA-2 = 1.01 µg/ml - BST LC_{50} = 0.31 µg/ml	Alali et al., 1997a
(2-4 *cis* and *trans*)-Gonioneninone				
	*G. giganteus*	(bark)	- Cytotoxic activity (IC_{50}) A-549 = 1.8; MCF-7 = 3.7; HT-29 = 2.9; A-498 = 2.2 x 10^{-1}; PC-3 = 1.5 and PACA-2 = 4.5 x 10^{-2} µg/ml - BST LC_{50} = 3.0 x 10^{-1} µg/ml	Alali et al., 1998a

Compound	Plant source	(part of plant)	Activity	Reference
Mono-tetrahydropyran				
Pyranicin  $R=(CH_2)_{11}CH_3$	*G. giganteus*	(bark)	- Cytotoxic activity (IC_{50}) A-549 = 2.8 x 10^{-1}; MCF-7 = 3.9 x 10^{-1}; HT-29 = 1.2; A-498 = 1.8 x 10^{-1}; PC-3 = 4.1 x 10^{-1} and PACA-2 = 1.3 x 10^{-3} µg/ml - BST LC_{50} = 0.3 µg/ml	Alali et al., 1998b
Pyragonicin  $R=(CH_2)_{13}CH_3$	*G.giganteus*	(bark)	- Cytotoxic activity (IC_{50}) A-549 = 2.0; MCF-7 = 1.6; HT-29 = 2.8; A-498 = 1.3; PC-3 = 1.2 and PACA-2 = 5.8 x 10^{-2} µg/ml - BST LC_{50} = 0.9 µg/ml	Alali et al., 1998b

Compound	Plant source	(part of plant)	Activity	Reference
Non adjacent bis-THF				
(2,4-*cis* and *trans*)-Gigantecinone 	*G. giganteus*	(bark)	- Cytotoxic activity (IC_{50}) A-549 = 2.14 x 10^{-1}; MCF-7 > 1; HT-29 > 1; A-498 = 2.12 x 10^{-1}; PC-3 = 1.08 x 10^{-3} and PACA-2 > 1 µg/ml - BST LC_{50} = 3.27 µg/ml	Alali et al., 1997b
Deoxygigantecin 	*G. giganteus*	(bark)	- Cytotoxic activity (IC_{50}) A-549 = 1.31 x 10^{-1}; MCF-7 = 1.0; HT-29 = 1.43 x 10^{-1}; A-498 = 3.28 x 10^{-1}; PC-3 = 1.50 x 10^{-1} and PACA-2 = 3.93 x 10^{-1} µg/ml - BST LC_{50} = 0.04 µg/ml	Alali et al., 1997b

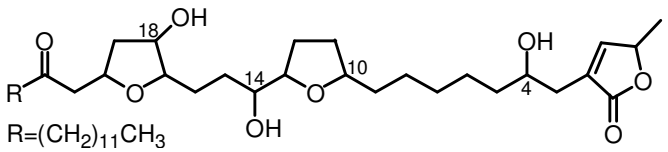
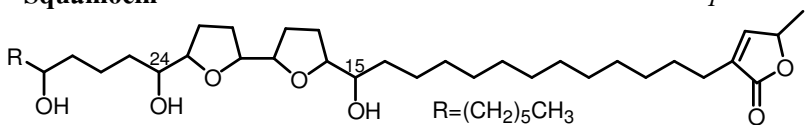
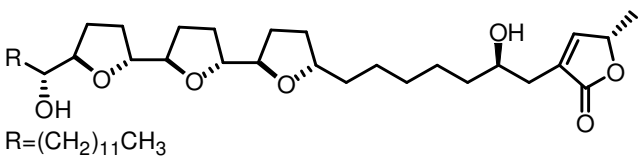
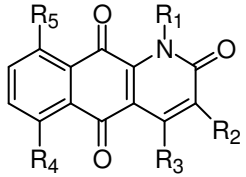
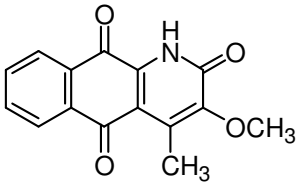
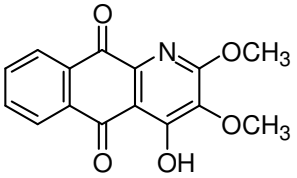
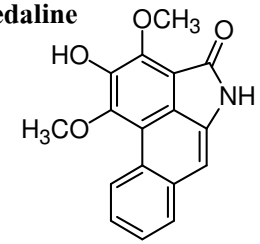
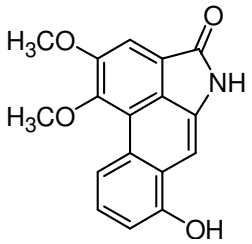
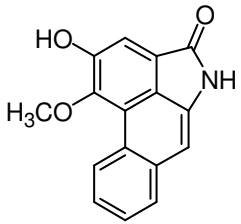
Compound	Plant source	(part of plant)	Activity	Reference
Goniotricin				
	*G. giganteus*	(bark)	- Cytotoxic activity (IC_{50}) A-549 = 3.3 x 10^{-2}; MCF-7 = 3.3 x 10^{-5}; HT-29 = 1.2 x 10^{-3}; A-498 = 1.1; PC-3 = 2.6 x 10^{-1} and PACA-2 = 1.4 µg/ml - BST LC_{50} = 2.7 µg/ml	Alali et al., 1999
Adjacent bis-THF				
Squamocin 	*G. cardiopetalus*	(stem barks)	- Cytotoxic activity (IC_{50}) L1210 = 0.58 µg/ml	Rupprecht et al., 1990; Hisham *et al*., 2003
Tri-THF				
Goniocin 	*G. gigantues*	(barks)	- Cytotoxic activity (IC_{50}) A-549 = 9.42 x 10^{-1}; MCF-7 = 4.85 and HT-29 = 1.61 x 10^{-2} µg/ml - BST LC_{50} = 57 µg/ml	Gu et al., 1994b

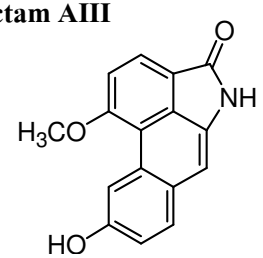
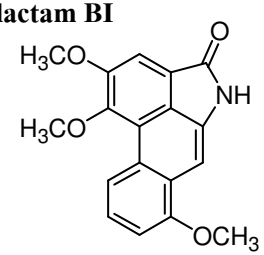
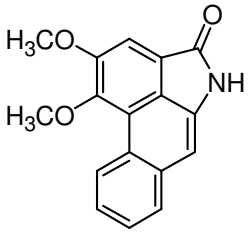
Table 4: Alkaloids from *Goniothalamus* spp.

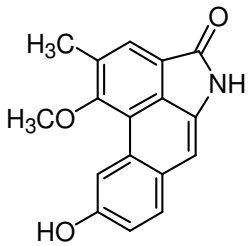
Compound	Plant source (part of plant)	Activity	Reference
Aza-anthraquinone			
Marcanine A-E  	*G. marcanii* (stem bark)	**Marcanine A** - Cytotoxic activity (IC_{50}) A-549 = 0.42; HT-29 = 0.42; MCF-7 = 0.42; RPMI = 0.42 and U251 = 0.84 µM **Marcanine B** - Cytotoxic activity (IC_{50}) A-549 = 0.35; HT-29 = 2.12; MCF-7 = 0.18; RPMI = 0.70 and U251 = 1.40 µM **Marcanine C** - Cytotoxic activity (IC_{50}) A-549 = 1.00; HT-29 = 0.33; MCF-7 = 1.00; RPMI = 0.67 µM and U251 < 10 µg/ml	Soonthornchareonnon et al., 1999

	R_1	R_2	R_3	R_4	R_5
A;	H	H	CH_3	H	H
B;	CH_3	OCH_3	CH_3	H	H
C;	CH_3	OCH_3	CH_2OH	H	H
D;	H	OCH_3	CH_3	OH	H
E;	CH_3	OCH_3	CH_3	H	OH

Compound	Plant source	(part of plant)	Activity	Reference
			Marcanine D - Cytotoxic activity (IC_{50}) A-549 = 0.04; HT-29 = 0.35; MCF-7 = 0.08; RPMI = 0.08 and U251 = 0.28 µM	
Dielsiquinone 	*G. marcanii*	(stem bark)	- Cytotoxic activity (IC_{50}) A-549 = 0.42; HT-29 = 0.42; MCF-7 = 0.42; RPMI = 0.42 and U251 = 0.84 µM	Soonthornchareonnon et al., 1999
Scorazanone 	*G. scortechinii*	(root)	-	Din et al., 1990

Compound	Plant source	(part of plant)	Activity	Reference
Phenanthrene lactam				
Goniopedaline 	*G. sesquipedalis*	(leaf and twig)	-	Talapatra et al., 1988
Velutinam 	*G. amuyon* *G. griffithii* *G. tenuifolius* *G. velutinus*	(stem) (root) (bark) (bark)	- Cytotoxic activity (IC_{50}) HeLa = 0.39 and L1210 = 1.16 mg/ml - Antimalarial activity	Omar et al., 1992; Likhitwitayawuid et al., 1997; Zhang et al., 1999; Lan et al., 2006
Aristolactam AII 	*G. griffithii* *G. tenuifolius*	(root) (bark)	- Antimalarial activity	Likhitwitayawuid et al., 1997; Zhang et al., 1999

Compound	Plant source	(part of plant)	Activity	Reference
Aristolactam AIII 	*G. borneensis*	(bark)	- Cytotoxic activity (IC_{50}) P388 = 5.2; WEHI164 = 24; THP-1 = 29.5 and MOLT4 = 4.3 µg/ml	Cao et al., 1998
Aristolactam BI 	*G. griffithii* *G. tenuifolius*	(root) (bark)	- Antimalarial activity	Likhitwitayawuid et al., 1997; Zhang et al., 1999
Aristolactam BII 	*G. amuyon* *G. cheliensis* *G. griffithii* *G. tenuifolius* *G. velutinus*	(stem) (root bark) (root) (bark) (bark)	- Cytotoxic activity (IC_{50}) HeLa = 3.77 and L1210 = 3.92 mg/ml - Antimalarial activity	Omar et al., 1992; Likhitwitayawuid et al., 1997; Zhang et al., 1999; Lan et al., 2006; Jiang et al., 2008

Compound	Plant source	(part of plant)	Activity	Reference
Goniothalactam				
	*G. borneensis*	(bark)	- Cytotoxic activity (IC_{50}) P388 > 30 µg/ml - as inhibitor of platelet aggregation induced by AA and collagen (% aggregation 13.6 ± 0.9, 10.5 ± 2.7 respectively at concentration 100 µg/ml)	Cao et al., 1998

1.3 *Goniothalamus undulatus* Ridl.

Distribution

G. undulatus, is a small tree (4-6 m high) which distributed in evergreen forests in Peninsular Thailand (Krabi, Nakhon Si Thammarat, Phattalung, Phuket, Ranong, Surat Thani and Trang Province) (Figure 3) (Saunders and Chalermglin, 2008).

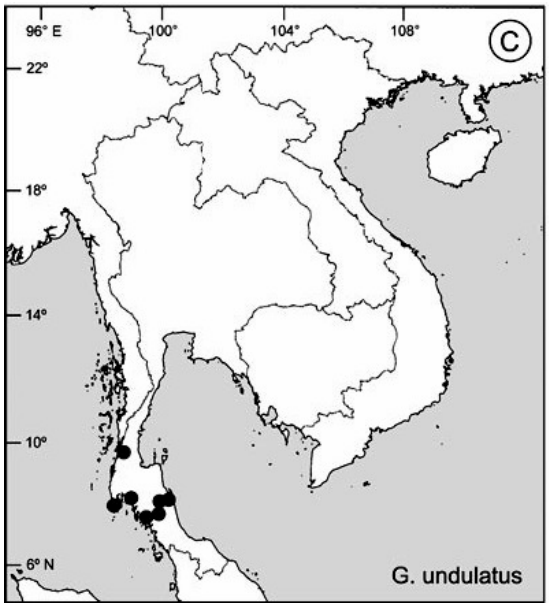


Figure 3: Distribution of *G. undulatus* in Thailand (Saunders and Chalermglin, 2008)

Taxonomic characteristics

Leaves coriaceous large (3-5 cm wide, 10-16 cm long), simple, alternate and exstipulate. Adaxial surface of leaves with slightly prominent secondary and tertiary vains. The blade is glossy, oblong lanceolate and thick. Leaves margin undulate. Petioles 5-8 mm long. The flowers are axillary and have two whorls of petal. Pedicels 7-23 mm long. Outer petals 5.5-17.5 mm wide and 10.5-32 mm long. Inner petal (8-10 mm long) claws with distinct glabrous lateral flange. Capels 10-54 per flower. Sepals thick and ovate. The fruits are apocarpous with individual

monocarp. Fruiting pedicels are ≥ 12 mm long and monocarps distinctly stipitate with stipe 6.5-16 mm long (Saunders and Chalermglin, 2008).

Figure 4: *Goniothalamus undulatus* Ridl.

Many species in genus *Goniothalamus* have been studied about chemical constituents and biological activities. Cytotoxic compounds have been found abundantly in this genus.

The preliminary study of cytotoxic property of *Goniothalamus* found in southern Thailand, four species of *Goniothalamus* were collected including *G. macrophyllus*, *G. tapis*, *G. giganteus* and *G. undulatus*. Methanolic extract of leaves, twigs and roots of four species were subjected to SRB cytotoxicity assay against three types of cancer cell lines (HepG2, MCF-7 and COR-L23). The result showed that the roots extract of *G. undulatus* exhibited the highest cytotoxicity against HepG2 (IC_{50} < 1µg/ml) and MCF-7 (IC_{50} < 1µg/ml) but less activity against COR-L23 (IC_{50} = 7.54µg/ml).

Although, *Goniothalamus spp.* have been investigated, there is no report for *G. undulatus*.

1.4 Objectives

The aims of this study are

(1) to investigate cytotoxic activity of the *G. undulatus* extracts by SRB assay,

(2) to study and isolate chemical constituents from *G. undulatus* extract and

(3) to evaluate the cytotoxic activity of isolated compounds.

CHAPTER 2

EXPERIMENTAL

2.1 General experimental procedures

^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectra were recorded on a Varian Unity Inova 500 MHz NMR spectrometer by using $CDCl_3$ as solvent. Mass spectra were measured on a Waters 2690-LCT Alliance-Micromass and Thermofinnigan MAT 95 XL mass spectrometers. IR spectra were recorded on a Jasco IR-810 infared spectrometer. UV spectra were obtained in methanol on a Hewlett Packard 8452A diode array spectrophotometer. The optical rotations were determined on a Jasco P-120 polarimeter. High performance liquid chromatography (HPLC) separations were performed with a Waters 600E multisolvent delivery system. This was connected to a Waters 484 tunable absorbance detector and to a Rheodyne 7125 injector port. Silica gel 60 (Merck, 40-63 μm) and Sephadex® LH-20 (Sigma-Aldrich, 25-100 μm) were used for column chromatography, while thin-layer chromatography (TLC) was performed on Merck silica gel 60 F_{254} aluminium sheet (250 μm thickness) and spots were detected by UV absorption and spraying with anisaldehyde reagent followed by heating the plates.

2.1 Plant material

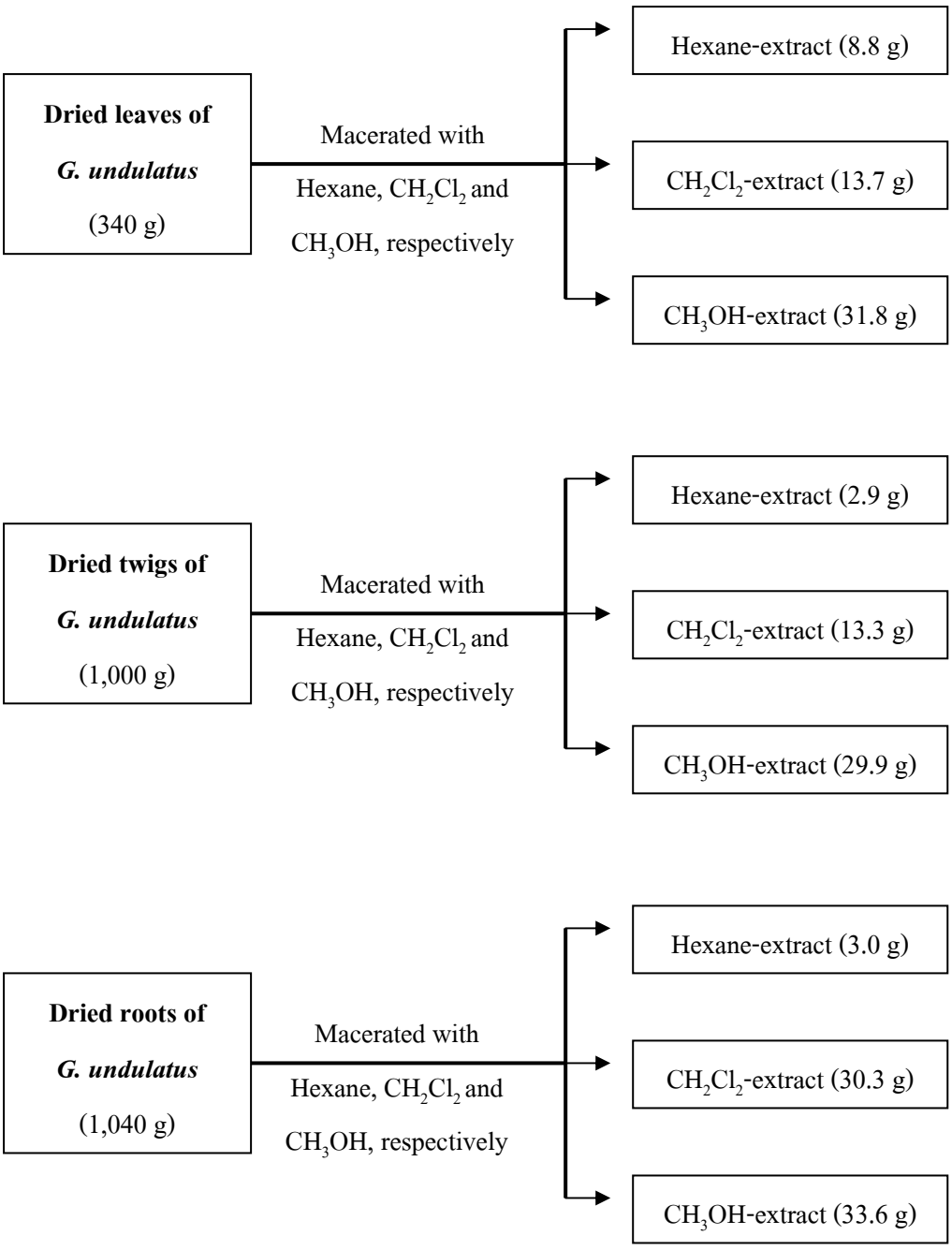
Leaves, twigs and roots of *G. undulatus* were collected from Khao Poo - Khao Ya National Park situated in Si Banpot District, Phatthalung Province, Thailand, in October 2006. The specimen was identified by Asst. Prof. Dr. Chatchai Wattanapiromsakul, Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University and confirmed by Asst. Prof. Dr. Choathip Purintavaragul, Department of Biology, Faculty of Sciences, Prince of Songkla University, Hat Yai, Songkhla, Thailand.

(a) (b) (c)

Figure 5: Leaves (a), twigs (b) and roots (c) of *G. undulatus*

2.3 Preparation of plant extracts

Each part of plant was washed, cut, oven-dried at 50°C and ground to powder. The powdery leaves (0.34 kg), twigs (1.00 kg) and roots (1.04 kg) were extracted with hexane by maceration method and were extracted repeatedly with dichloromethane and methanol, respectively. The solvents from extraction were removed by rotary evaporation to obtain nine crude extracts from leaves, twigs and roots (Scheme 1).



Scheme1: Extraction scheme of each part of *G. undulatus*

2.4 *In vitro* assay for cytotoxic activity

2.4.1 Cell lines

The extracts were investigated their cytotoxic activity against three types of cancer cell lines; large cell lung carcinoma COR-L23, human breast adenocarcinoma MCF-7 (ECACC No: 86012803) and human hepatocellular liver carcinoma HepG2 and one type of normal cell line; human fetal fibroblast cell line MRC-5. COR-L23 cells were established and kindly provided by Dr. P. Twentyman and Dr. P. Rabbitts of MRC Clinical Oncology & Radiotherapeutics Unit, Cambridge, UK and were cultured in RPMI 1640 medium supplement with 10% heated foetal bovine serum, 1% of 2 mM L-glutamine, 50 IU/ml penicillin and 50 µg/ml streptomycin. MCF-7 cells were obtained from the European Collection of Animal Cell Culture (PHLS Center for Applied Microbiology Research, Porton Down, Salisbury, UK) were cultured in Minimum Essential Media (MEM) with Earle Salt (without glutamine medium supplement) with 10% heated foetal bovine serum, 1% of 2 mM L-glutamine, 50 IU/ml penicillin and 50 µg/ml streptomycin and 1% non-essential amino acid. HepG2 cells were cultured in MEM with 10% heated foetal bovine serum, 1% of 2 mM L-glutamine, 50 IU/ml penicillin and 50 µg/ml streptomycin and 0.3% HEPES. MRC-5 cells were cultured with MEM with 10% heated foetal bovine serum, 1% of 2 mM L-glutamine, 50 IU/ml penicillin and 50 µg/ml streptomycin and maintained at 37°C in a 5% CO_2 atmosphere with 95% humidity (Keawpradub et al., 1999). The optimal plating densities of each cell line were determined ($1x10^3$, $3x10^3$, $3x10^3$and $5x10^3$ cells/well for COR-L23, MCF-7, HepG2 and MRC-5, respectively) to ensure exponential growth throughout the experimental period and to ensure a linear relationship between absorbance at 492 nm and cell number when analysed by SRB assay.

2.4.2 Cytotoxicity assay

In the beginning, cells were washed with phosphate buffer saline (PBS) (Oxoid Ltd., UK) free of magnesium and calcium. The PBS was decanted and cells detached with

0.025% trypsin-EDTA (Sigma-Aldrich) and PBS was added to a volume of 50 ml. The cell pellet, obtained by centrifugation was resuspended in 10 ml of medium to make a single cell suspension. Viable cells density being counted by trypan blue exclusion in a heamocytometer and then diluted with medium to give the previously-determined optimal plating densities for each cell type. One hundred µl/well of these cell suspensions were seeded in 96-well microtiter plates and incubated at 37°C to allow for cell attachment. After 24 h the cells were treated with the extracts or pure compounds. Each extract was dissolved in dimethylsulfoxide. Vincristine sulphate (Sigma-Aldrich) was used as positive control. Fifty µg/ml of each extract was tested initially against all three cancer cell lines. From these results, the active extracts were considered to be those which gave less than 20% survival at exposure time 72 h. These extracts were investigated cytotoxicity with cell lines and calculated IC_{50} values. Each active extracts was diluted in medium to produce 8 concentrations of 0.1, 0.5, 1, 5, 10, 25, 50, 100 µg/ml. One hundred µl/well of each concentration was added to the plates in 6 replicates. The final dilution used for treating the cells contained not more than 1% of the initial solvent, this concentration being used in the solvent control wells. The plates were incubated for the selected exposure time of 72 h. At the end of each exposure time, the medium was removed and the wells were washed with medium and 200 µl of fresh medium were then added. The plates were incubated at 37 °C for a recovery period of 6 days and cell growth was then analysed using the SRB assay. Three replicate plates were used to determine the cytotoxicity of each extract.

2.4.3 Sulphorhodamine B (SRB) assay (Skehan et al., 1990)

This assay was performed to assess growth inhibition by colorimetric assay which estimates cell number indirectly by staining total cellular protein with SRB (Skehan et al., 1990). In brief, cells were fixed by layering 100 µl of ice-cold 40% trichloroacetic acid (TCA), (Merck) on top of the growth medium. Cells were incubated at 4ºC for 1 h., after which plates were washed five times with cold water, the excess water was drained off and the plates were left to dry in air. SRB stain (50 µl; 0.4% in 1% acetic acid) (Sigma) was added to each well and left in contact with the cells for 30 minutes, after which they were washed with 1% acetic acid, rinsed 4 times until only dye adhering to the cells was left. The plates were dried and 100 µl of 10 mM

Tris base pH 10.5 (Sigma-Aldrich) were added to each well to solubilise the dye. The plates were shaken gently for 20 minutes on a gyratory shaker and the absorbance (OD) of each well was read on a SLT 340 ATTC plate reader (SLT Lab instrument, Australia) at 492 nm. Cell survival was measured as the percentage absorbance compared to the control (non-treated cells). The IC_{50} values were calculated from the Anilisa program by plotting the percentage survival versus the concentrations, interpolated by cubic spine. According to National Cancer Institute guidelines (Boyd, 1997) extracts with IC_{50} values < 20 µg/ml were considered active.

2.5 Isolation and purification

Dichloromethane extracts from roots showed high cytotoxic activity and more selective against cancer cell lines than normal cell line and highest obtainable mass, therefore this extract was isolated for isolation and purification by chromatographic techniques. In beginning, the extract was isolated with vacuum chromatography over a silica gel column, eluted with step-gradient solvents starting from hexane to 20% ethyl acetate in methanol. The eluents were combined into 7 fractions (F1-F7) on the basic of TLC. All of fractions were investigated cytotoxic activity against cancer cell lines (MCF-7 and COR-L23). F4 and F5 showed the most active against COR-L23 and MCF-7, respectively (IC_{50}= 2.19 and 0.58 µg/ml). While F7 showed good cytotoxic activity (IC_{50} = 3.27 (MCF-7) and 6.82 (COR-L23) µg/ml). These fractions were selected for the further isolation. The isolation scheme of dichloromethane extract from roots show in Scheme 2.

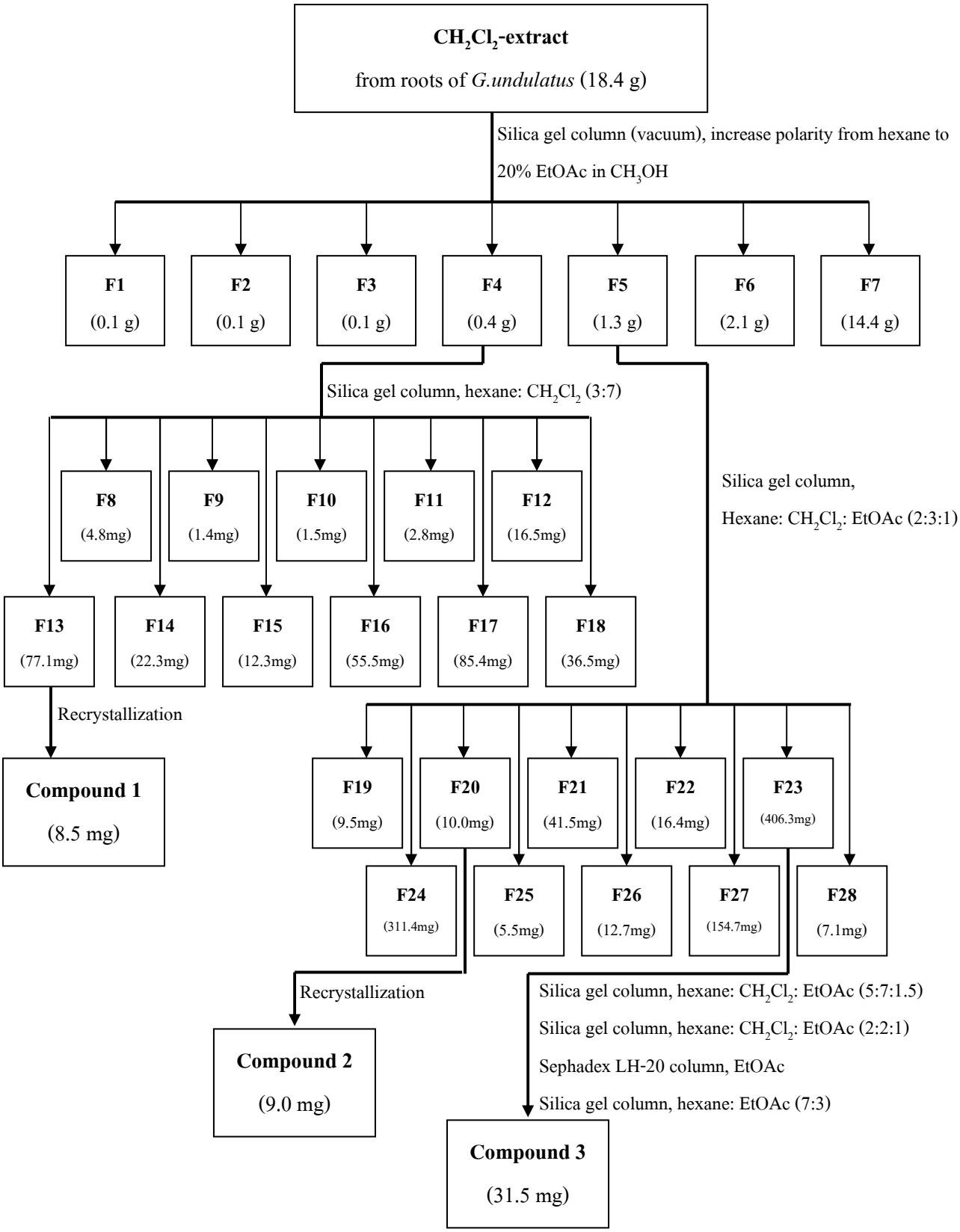
F4 was separated using silica gel column chromatography eluted with hexane: dichloromethane (3:7) to give F8-F18. Compound 1 (8.5 mg) was isolated from F13 by recrystallization with dichloromethane.

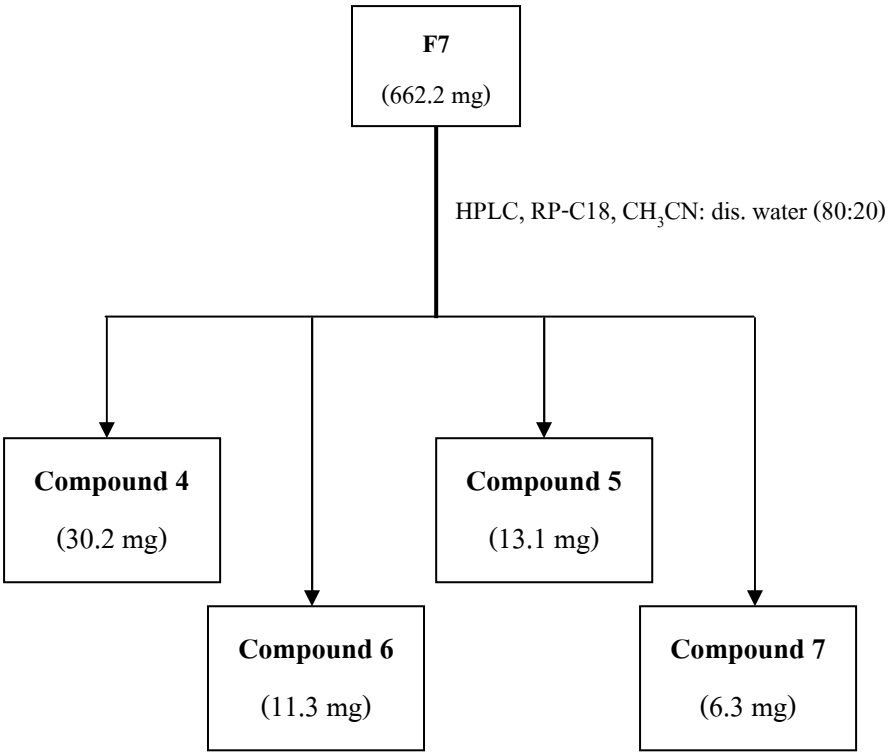
F5 was separated by silica gel column chromatography (hexane: dichloromethane: ethyl acetate, 2:3:1) to give 10 fractions (F19-F28). F20 was purified by recrystallization to give compound 2 (9 mg).

F23 was separated according to high obtainable mass by silica gel column chromatography with solvent system as hexane: dichloromethane: ethyl acetate (5:7:1.5) to give F29-F33. Column chromatography of F33 with hexane: dichloromethane: ethyl acetate (2:2:1),

eluted over silica gel, was separated. The eluents were combined into 6 fractions (F34-F39). F37 was isolated and purified by Sephadex LH-20 (100% ethyl acetate) and silica gel (hexane: ethyl acetate, 7:3) column chromatography to give compound 3 (31.5 mg)

Compound 4-7 (30.2, 13.1, 11.3 and 6.3 mg, respectively) were isolated from F7 using HPLC RP-C18 column (Phenomenex®, 10 μm, 250 x 10 mm; 20% distilled water in acetonitrile, flow rate 3.0 ml/min). Retention times of each compound were 23.6, 24.7, 31.8 and 33.1 min, respectively.


CH2Cl2-extract
from roots of G.undulatus (18.4 g)
Silica gel column (vacuum), increase polarity from hexane to 20% EtOAc in CH3OH
F1 (0.1 g)
F2 (0.1 g)
F3 (0.1 g)
F4 (0.4 g)
F5 (1.3 g)
F6 (2.1 g)
F7 (14.4 g)
Silica gel column, hexane: CH2Cl2 (3:7)
F8 (4.8mg)
F9 (1.4mg)
F10 (1.5mg)
F11 (2.8mg)
F12 (16.5mg)
F13 (77.1mg)
F14 (22.3mg)
F15 (12.3mg)
F16 (55.5mg)
F17 (85.4mg)
F18 (36.5mg)
Recrystallization
Compound 1 (8.5 mg)
Silica gel column, Hexane: CH2Cl2: EtOAc (2:3:1)
F19 (9.5mg)
F20 (10.0mg)
F21 (41.5mg)
F22 (16.4mg)
F23 (406.3mg)
F24 (311.4mg)
F25 (5.5mg)
F26 (12.7mg)
F27 (154.7mg)
F28 (7.1mg)
Recrystallization
Compound 2 (9.0 mg)
Silica gel column, hexane: CH2Cl2: EtOAc (5:7:1.5)
Silica gel column, hexane: CH2Cl2: EtOAc (2:2:1)
Sephadex LH-20 column, EtOAc
Silica gel column, hexane: EtOAc (7:3)
Compound 3 (31.5 mg)



Scheme 2: Isolation scheme of CH_2Cl_2-extract from roots of *G. undulatus*

2.6 Physical properties of isolated compounds

Compound 1: **5-Acetoxyisogoniothalamin oxide**; transparent needles; $[\alpha]_D$ +89.3 ($CHCl_3$: *c* 0.21); UV (MeOH) λ_{max} 210 nm; IR (neat) ν_{max} 2920, 1740, 1710, 1370, 1220 cm^{-1}; ^1H and ^{13}C NMR see Table 9; ESIMS *m/z* (% relative intensity) 297 ($[M+Na]^+$, 100).

Compound 2: **O-Acetylaltholactone**; transparent needles; $[\alpha]_D$ +167.4 ($CHCl_3$: *c* 0.11); UV (MeOH) λ_{max} 208 nm; IR (neat) ν_{max} 1750, 1730, 1220, 1100, 1050, 700 cm^{-1}; ^1H and ^{13}C NMR see Table 8; ESIMS *m/z* (% relative intensity) 297 ($[M+Na]^+$, 100).

Compound 3: **Altholactone**; yellowish oil; $[\alpha]_D$ +149.3 ($CHCl_3$: *c* 0.81); UV (MeOH) λ_{max} 208 nm; IR (neat) ν_{max} 3400, 1710, 1240, 1095, 690 cm^{-1}; ^1H and ^{13}C NMR see Table 7; ESIMS *m/z* (% relative intensity) 233 ($[M+H]^+$, 100).

Compound 4: **Annonacin**; white amorphous wax; $[\alpha]_D$ +21.5 ($CHCl_3$: *c* 0.20); UV (MeOH) λ_{max} 212 nm; IR (neat) ν_{max} 3430, 2900, 2840, 1730, 1450, 1310, 1060 cm^{-1}; ^1H and ^{13}C NMR see Table 10; ESIMS *m/z* (% relative intensity) 619 ($[M+Na]^+$, 100); EIMS spectrum see Figure 15.

Compound 5: ***cis*-Annonacin**; white amorphous wax; $[\alpha]_D$ +8.2 ($CHCl_3$: *c* 0.19); UV (MeOH) λ_{max} 212 nm; IR (neat) ν_{max} 3400, 2900, 2840, 1740, 1450, 1310, 1070 cm^{-1}; ^1H and ^{13}C NMR see Table 11; ESIMS *m/z* (% relative intensity) 619 ($[M+Na]^+$, 100); EIMS spectrum see Figure 19.

Compound 6: **Goniothalamicin**; white amorphous wax; $[\alpha]_D$ +13.0 ($CHCl_3$: *c* 0.16); UV (MeOH) λ_{max} 210 nm; IR (neat) ν_{max} 3440, 2910, 2840, 1730, 1460, 1310, 1080, 1020 cm^{-1}; ^1H and ^{13}C NMR see Table 12; ESIMS *m/z* (% relative intensity) 619 ($[M+Na]^+$, 100), 597 ($[M+H]^+$, 45); EIMS spectrum see Figure 23.

Compound 7: ***cis*-Goniothamicin**; white amorphous wax; $[\alpha]_D$ +8.3 ($CHCl_3$: *c* 0.12); UV (MeOH) λ_{max} 210 nm; IR (neat) ν_{max} 3380, 2910, 2840, 1730, 1460, 1310, 1070, 1020 cm^{-1}; ^1H and ^{13}C NMR see Table 12; ESIMS *m/z* (% relative intensity) 619 ($[M+Na]^+$, 100); EIMS spectrum see Figure 27.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Cytotoxic activity of *G. undulatus* extracts

Each part of *G. undulatus* was extracted and investigated cytotoxic activity. Weight and percentage yield of *G. undulatus* extracts are shown in Table 5.

Table 5: Weight and % yield of *G. undulatus* extracts

Part of plant	Extract	Weight (g)	% Yield
Leaves	Hexane	8.78	2.58
	Dichloromethane	13.73	4.04
	Methanol	31.78	9.35
Twigs	Hexane	2.92	0.29
	Dichloromethane	13.34	1.33
	Methanol	29.94	2.99
Roots	Hexane	3.04	0.29
	Dichloromethane	30.34	2.92
	Methanol	33.58	3.23

The active extracts were considered to be those which gave less than 20% survival at exposure time 72 h. Percentage survival of cancer cell lines treated with extract concentration 50µg/ml are shown in Figure 6. This data showed that dichloromethane extracts from leaves, twigs and roots exhibited high cytotoxic activity against COR-L23, MCF-7 and HepG2 where the percentage survival of cancer cell lines were 0.58-2.37.

Active extracts were investigated for their cytotoxicity in cell lines and calculated IC_{50} values. The results are shown in Table 6.

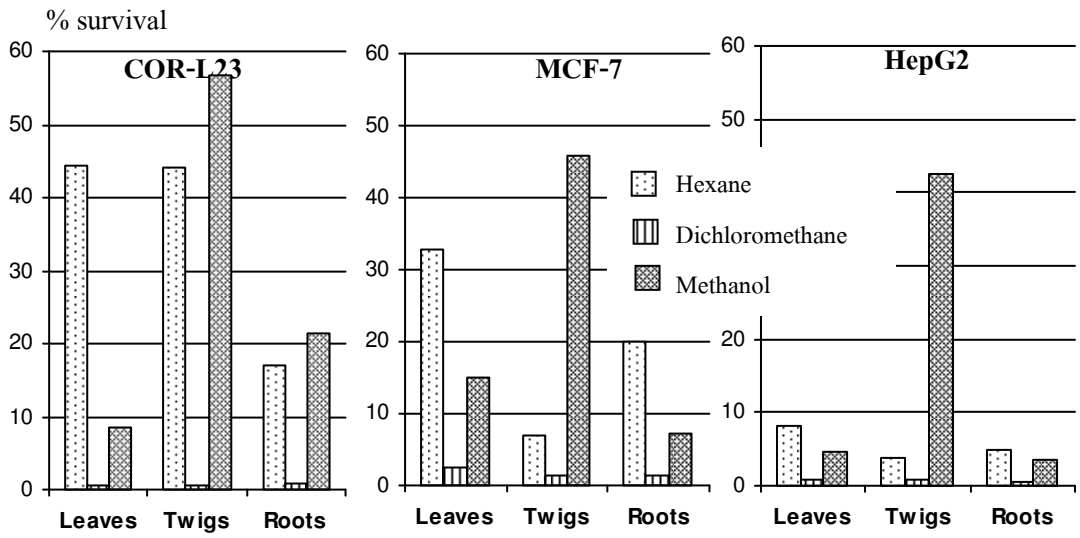


Figure 6: Percentage survival of cancer cell lines (COR-L23, MCF-7 and HepG2) treated with extract concentration 50µg/ml at exposure time 72 h (n=2)

Table 6: Cytotoxicity of *G. undulatus* extracts (IC_{50} (µg/ml) ± S.E.M.) (n=4)

Part of plant	Extract	IC_{50} (µg/ml) ± S.E.M.			
		COR-L23	**MCF-7**	**HepG2**	**MRC-5**
Leaves	Hexane	NT	NT	4.64 ± 0.50*	67.58 ± 2.20
	Dichloromethane	3.01 ± 0.85	0.27 ± 0.04	0.28 ± 0.03	24.28 ± 1.02
	Methanol	10.48 ± 1.85	3.55 ± 1.31	0.46 ± 0.02	86.47 ± 1.83
Twigs	Hexane	NT	7.12 ± 0.99	0.84 ± 0.22	> 100
	Dichloromethane	4.14 ± 0.63	0.26 ± 0.01	0.06 ± 0.03	24.83 ± 1.59
	Methanol	NT	NT	NT	> 100
Roots	Hexane	13.70 ± 1.83*	2.44 ± 0.37*	0.47 ± 0.08	86.79 ± 2.51
	Dichloromethane	2.53 ± 0.70	1.76 ± 0.29*	0.15 ± 0.03*	29.12 ± 0.68
	Methanol	NT	3.70 ± 0.87*	0.36 ± 0.05	67.28 ± 2.19

* n=3, NT: not tested (Each extract at 50 µg/ml concentration gave more than 20% survival)

Lung cancer cells were treated with five extracts from three parts of *G. undulatus*. Dichloromethane extracts from leaves, twigs and roots showed the most cytotoxic

activity against COR-L23. The IC_{50} values were 3.01, 4.14 and 2.53 µg/ml respectively. Seven extracts were investigated for their cytotoxicities against MCF-7. The data showed all three dichloromethane extracts had high cytotoxicity and the IC_{50} values were 0.27, 0.26 and 1.76 µg/ml (leaves, twigs and roots extracts, respectively). All extracts showed good cytotoxic activity against hepatocyte cells. The IC_{50} values were less than 1 µg/ml except hexane extract of leaves while dichloromethane extracts from leaves, twigs and roots exhibited highest effect. The IC_{50} values were less than 0.3µg/ml.

For their effects on normal cell line, all of extracts showed IC_{50} values above 20µg/ml against MRC-5. However, three dichloromethane extracts were more toxic. The IC_{50} values of leaves, twigs and roots extracts were 24.28, 24.83 and 29.12 µg/ml respectively.

These results showed that dichloromethane extracts from leaves, twigs and roots of this plant exhibited the highest cytotoxicity against COR-L23, MCF-7 and HepG2 and also showed more selective against cancer cell lines than normal cell line.

The dichloromethane extract of roots was selected for isolation of the chemical constituents according to its potent cytotoxicity and obtainable mass (30.34 g).

3.2 Isolation of active compounds

Dichloromethane extract of roots was separated by column chromatography technique. The extract (18.41 g) was subjected to silica gel vacuum column eluted with a gradient of hexane, ethyl acetate and methanol (100% hexane to 20% ethyl acetate in methanol). Three styryl lactones (compound 1-3) were further purified by silica gel and sephadex column chromatography. Compound 1-3 were obtained 8.5, 9.0 and 31.5mg, respectively. And four acetogenins (compound 4-7) were isolated using HPLC RP-C18 column. Compound 4-7 were obtained 30.2, 13.1, 11.3 and 6.3 mg, respectively.

3.3 The structure elucidation of isolated compounds

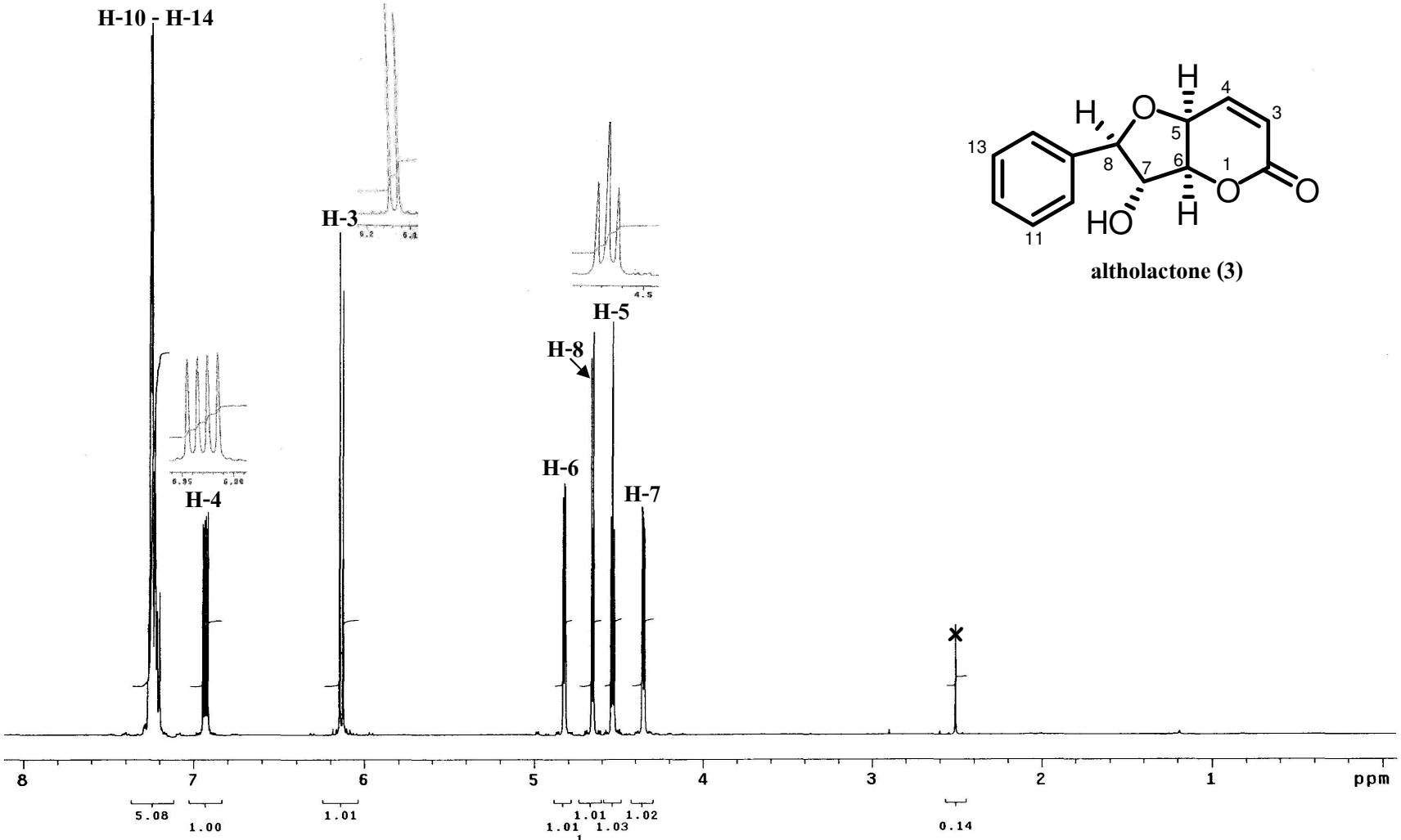
3.3.1 Styryl lactones (Compounds 1-3)

Compound 3 was isolated as yellow oil. The molecular formula of this compound was established as $C_{13}H_{12}O_4$ according to the $[M+H]^+$ peak at *m/z* 233 in low resolution ESIMS, in agreement with the 13 carbon atoms observed in the ^{13}C NMR (two quaternaries and eleven methines). The carbon signals at δ 128.1 (1C), 128.5 (2C), 126.1 (2C) and 138.3 (1C) and proton resonances at δ 7.25 (H), 7.22 (2H) and 7.24 (2H) in NMR experiments (Figures 7 and 8), corroborated the existence of a monosubstituted phenyl group.

The presence of one carbonyl group in 3 was established by carbon resonances at δ 161.5 (C-2) and by strong IR absorption at 1710 cm^{-1}.

The ^1H-^1H COSY spectrum showed correlation of an olefinic proton (H-4 at δ 6.93) to a vicinal oxygenated methane (H-5 at δ 4.54) and an olefinic proton (H-3 at δ 6.14), the coupling constants were 5 and 9.5 Hz, respectively, and consecutive proton correlation between remaining four oxygen bearing methines (H-5 to H-8). The coupling constants between H-5/H-6, H-6/H-7 and H-7/H-8 were reported to be 5, 2 and 5.5 Hz, respectively.

The HMBC spectrum (Table 7) showed correlation from C-2 to H-3, H-4, H-5 and H-6. Therefore, this fragment (C-2 to C-6) was suggested as an α,β-unsaturated δ-lactone moiety. The phenyl group was connected to C-8 (δ 86.2) according to the correlations from C-8 (δ 86.2) to H-10 and H-14; C-9 (δ 138.3) to H-8; and C-10 and C-14 to H-8. The structure of 3 was proposed to be altholactone (Figure 7). The spectral data were confirmed based on the previous report by El-Zayat et al. (1985).



altholactone (3)

Figure 7: The structure and ^{1}H NMR spectrum of compound 3 (500 MHz in $CDCl_3$)

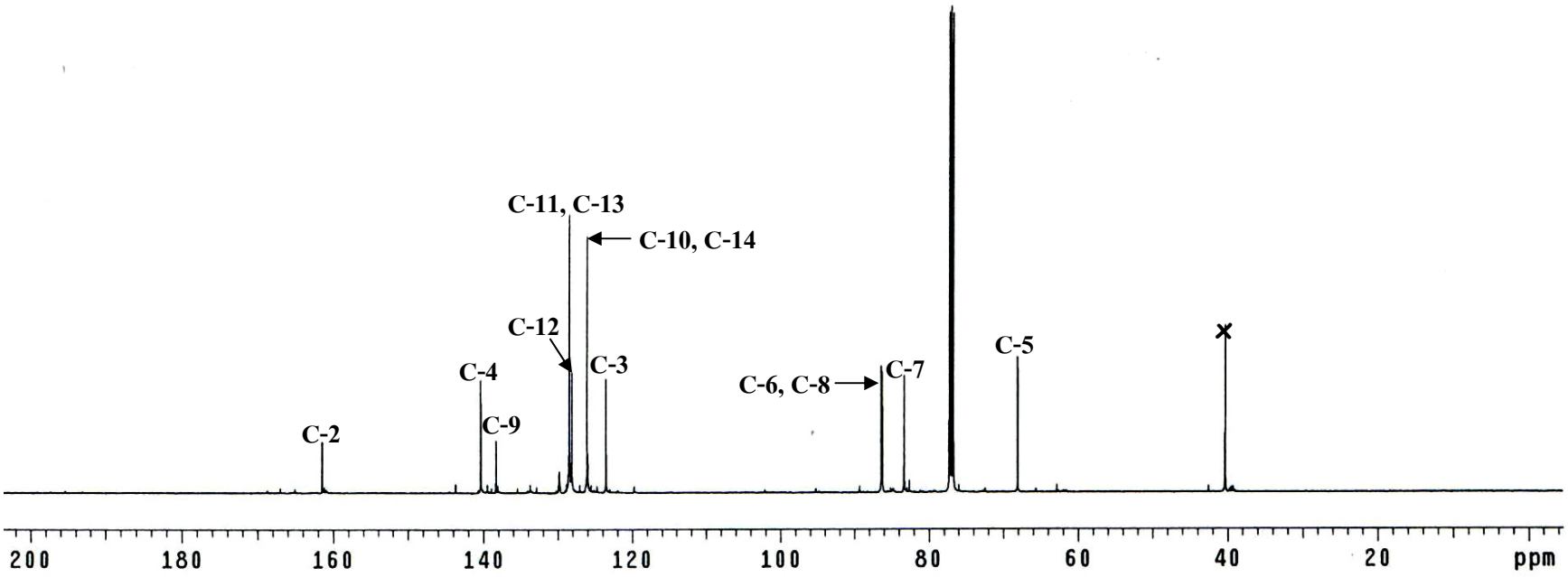


Figure 8: ^{13}C NMR spectrum of compound 3 (125 MHz in $CDCl_3$)

Table 7: NMR data of compound 3 (500 MHz for ^{1}H and 125 MHz for ^{13}C in $CDCl_3$)

Position	$\delta^{13}C$	(Type)	$\delta^{1}H$	mult. (*J* in Hz)	HMBC correlation (C►H)
2	161.5	C	-		H-3, H-4, H-5, H-6
3	123.6	CH	6.14	d (9.5)	H-5
4	140.4	CH	6.93	dd (9.5, 5.0)	H-5, H-6
5	68.1	CH	4.54	t (5.0)	H-3, H-4, H-6, H-7, H-8
6	86.4	CH	4.82	dd (5.0, 2.0)	H-4, H-5, H-8
7	83.3	CH	4.35	dd (5.5, 2.0)	H-6, H-8
8	86.2	CH	4.65	d (5.5)	H-6, H-7, H-10, H-14
9	138.3	C	-		H-7, H-8, H-10, H-11, H-13, H-14
10, 14	126.1	CH	7.24	m	H-8, H-11, H-12, H-13
11, 13	128.5	CH	7.22	m	H-10, H-12, H-14
12	128.1	CH	7.25	m	H-10, H-11, H-13, H-14

Compound 2 was isolated as transparent needles. The spectral data closely related to compound 3. Molecular weight of compound 2 was indicated in the low resolution ESIMS by the peak at *m/z* 297 $[M+Na]^{+}$, corresponding to the molecular formula $C_{15}H_{14}O_5$, which is in agreement with 15 carbon signals observed in the ^{13}C-NMR spectrum (three quaternaries, eleven methines and one methyl) (Figure 10).

Compound 2 showed carbon resonances at δ 160.4 and strong IR absorption at 1730 cm^{-1}, similar to compound 3 which suggests an α,β-unsaturated δ-lactone moiety. The ^{1}H-NMR spectrum (Figure 9) showed aromatic protons (δ 7.34 (2H) and 7.35 (3H)) and olefinic protons at δ 6.28 (d, *J*=9.5 Hz, 1H) and δ 7.04 (dd, *J*=5.2 and 9.5 Hz, 1H) which were assigned to be H-3 and H-4 respectively, while signals at δ 4.63 (dd, *J*=5.2 and 4.2 Hz, 1H), δ 4.95 (dd, *J*=4.2 and 0.7 Hz, 1H), δ 5.39 (d, *J*=3.0 Hz, 1H) and δ 4.98 (d, *J*=3.0 Hz, 1H) were assigned to be H-5, H-6, H-7 and H-8, respectively. The ^{1}H-NMR spectrum showed a singlet signal resonated at δ 2.16 (3H, H-OAc) belongs to the methyl of acetyl group. This is further supported by the ^{13}C NMR which showed carbonyl ester group at δ 169.4 and a methyl group at δ 20.8. These signals were not found in the NMR spectra of compound 3 (Table 8).

The HMBC spectrum showed correlation from carbon (δ 169.4) of acetyl group to H-7 and proton (δ 2.16, 3H) of acetyl group. Therefore, acetyl group was connected to C-7. The structure of compound 2 was suggested as O-acetylaltholactone which was confirmed base on spectral data and optical rotation from the previous report (Peris et al., 2000).

Table 8: NMR data of compound 2 (500 MHz for ^{1}H and 125 MHz for ^{13}C in $CDCl_3$)

Position	$\delta^{13}C$	(Type)	$\delta^{1}H$	mult. (*J* in Hz)	HMBC correlation (C►H)
2	160.4	C	-		H-3, H-4
3	124.7	CH	6.28	d (9.5)	H-5
4	139.1	CH	7.04	dd (9.5, 5.2)	H-5
5	69.1	CH	4.63	dd (5.2, 4.2)	-
6	83.6	CH	4.95	dd (4.2, 0.7)	H-4, H-5, H-7
7	83.5	CH	5.39	d (3.0)	H-5, H-6, H-8, H-OAc
8	86.1	CH	4.98	d (3.0)	H-6, H-10,H-11, H-12, H-13
9	137.4	C	-		H-8, H-10, H-11, H-12, H-13
10, 14	126.2	CH	7.34	m	H-8, H-11, H-12, H-13
11, 13	128.6	CH	7.35	m	H-10, H-12, H-14
12	128.5	CH	7.35	m	H-10, H-11, H-13, H-14
7-OAc	169.4, 20.8	C, CH_3	2.16	s	H-7, H-OAc

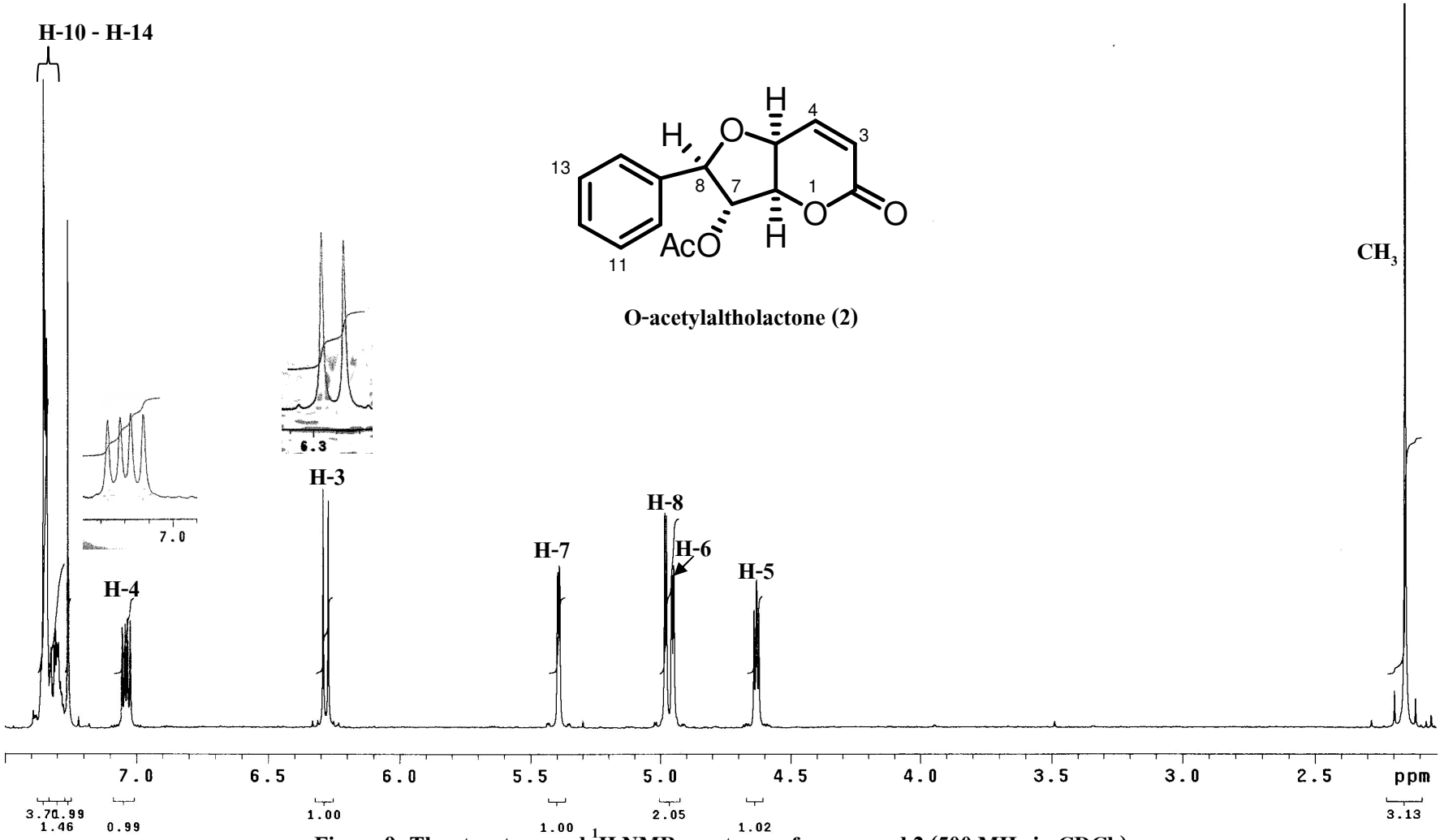


Figure 9: The structure and ^{1}H NMR spectrum of compound 2 (500 MHz in $CDCl_3$)

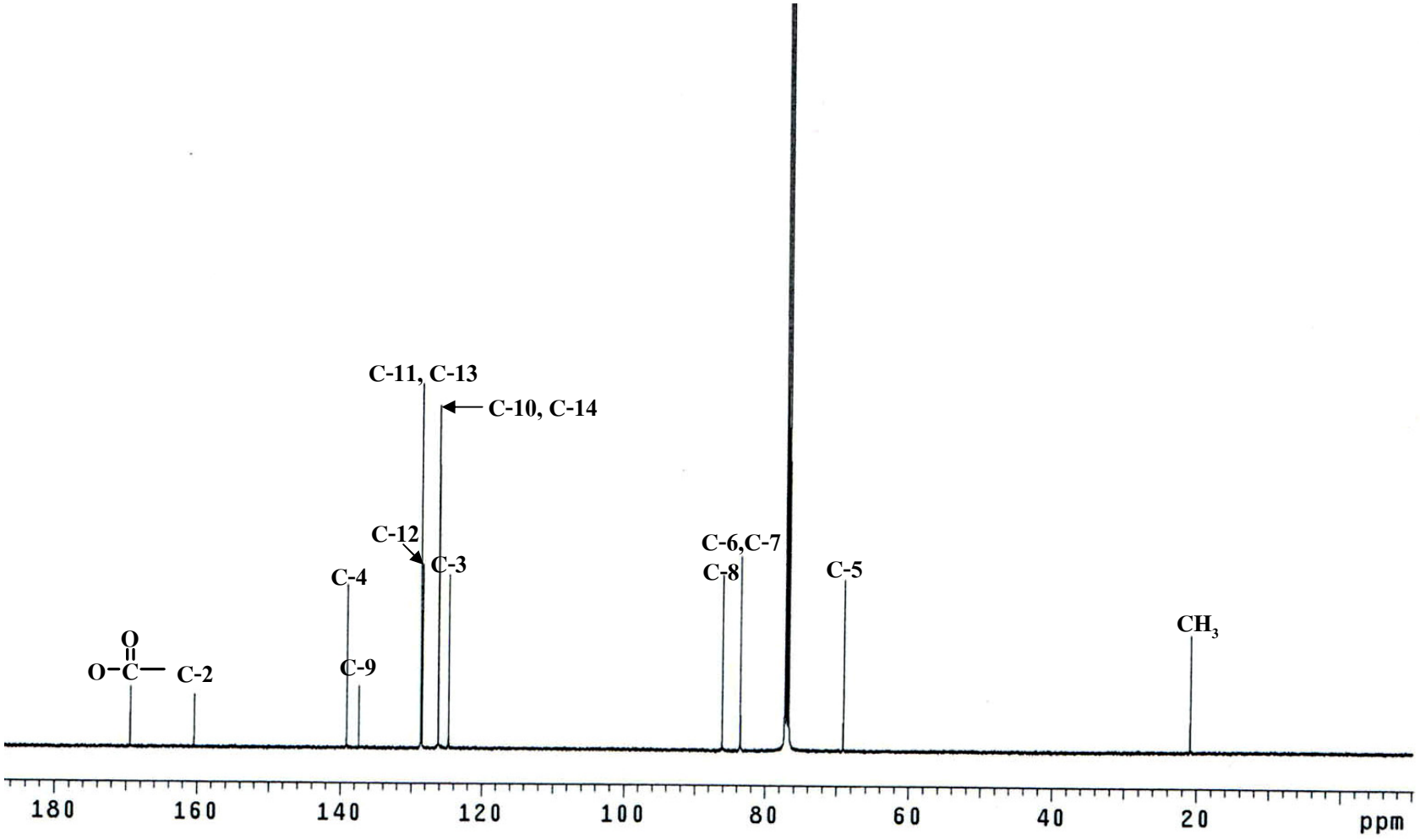


Figure 10: ^{13}C NMR spectrum of compound 2 (125 MHz in $CDCl_3$)

Compound 1 was isolated as transparent needles. The molecular formula of compound 1 was established as $C_{15}H_{14}O_5$ according to the $[M+Na]^+$ peak at *m/z* 297 in low resolution ESIMS, in agreement with the 15 carbon atoms observed in the ^{13}C NMR (three quaternaries, eleven methines and one methyl) (Figure 12).

The presence of two carbonyl groups in compound 1 was established by carbon signals at δ 162.0 (*α,β*-unsaturated *δ*-lactone, C-2) and δ 170.0 (acetyl group) and by two strong IR absorption at 1710 and 1740 cm^{-1}. And one methyl group was established by carbon signal at δ 20.5.

The ^1H NMR signals at δ 7.24 (3H) and 7.16 (2H) and ^{13}C NMR signals at δ 125.6 (2C), 128.6 (2C), 128.7 (1C), 135.4 (1C) represented a monosubstituted phenyl moiety. The spectra showed a deshielded proton attached to oxygenated carbon at δ 5.54 (dd, *J*=5.0 and 4.0 Hz, H-5) while the HMBC spectrum shows correlation from carbon (δ 170.0) of acetyl group to H-5 and proton of acetyl group (δ 1.95, s, 3H) (Table 9). Therefore, acetyl group was connected at C-5.

The ^1H-NMR spectrum (Figure 11) showed the olefenic protons at δ 6.14 (d, *J*=10.0 Hz, 1H) and 6.78 (dd, *J*= 10.0 and 5.0 Hz, 1H) were assigned to H-3 and H-4, respectively and the ^1H-^1H COSY spectrum showed consecutive proton correlation between four remaining oxygen bearing methines (H-5 to H-8) and ^1H-NMR spectrum revealed a disubstituted epoxide at δ 3.23 (dd, *J*=4.0 and 2.0 Hz, H-7) and δ 3.84 (d, *J*=2.0 Hz, H-8) (Sam et al., 1987). This evidence was supported by ^{13}C-NMR of C-7 (δ 59.5) and C-8 (δ 54.0), comparatively upfield when compared with C-7 and C-8 of its diol derivative. ^{13}C-NMR of C-7 and C-8 of goniodiol resonated at δ 73.6 and 75.0, repectively (Fang et al., 1991a).

The structure of compound 1 was proposed to be 5-acetoxyisogoniothalamin oxide (Figure 11) which was confirmed with the NMR experimental data from the previous report (Hasan et al*.,* 1994).

Table 9: NMR data of compound 1 (500 MHz for ^{1}H and 125 MHz for ^{13}C in $CDCl_3$)

Position	$\delta^{13}C$	(Type)	$\delta^{1}H$	mult. (*J* in Hz)	HMBC correlation (C►H)
2	162.0	C	-		H-3, H-4
3	124.7	CH	6.14	d (10.0)	H-5
4	139.8	CH	6.78	dd (10.0, 5.0)	H-5
5	62.9	CH	5.54	dd (5.0, 4.0)	H-3, H-4, H-6, H-OAc
6	77.0	CH	4.50	t (4.0)	H-4, H-7, H-8
7	59.5	CH	3.23	dd (4.0, 2.0)	H-6, H-8
8	54.0	CH	3.84	d (2.0)	H-7, H-10, H-14
9	135.4	C	-		H-8, H-10, H-11, H-12, H-13, H-14
10, 14	125.6	CH	7.16	m	H-8, H-11, H-12, H-13
11, 13	128.6	CH	7.24	m	H-10, H-12, H-14
12	128.7	CH	7.24	m	H-10, H-11, H-13, H-14
5-OAc	170.0, 20.5	C, CH_3	1.95	s	H-5, H-OAc

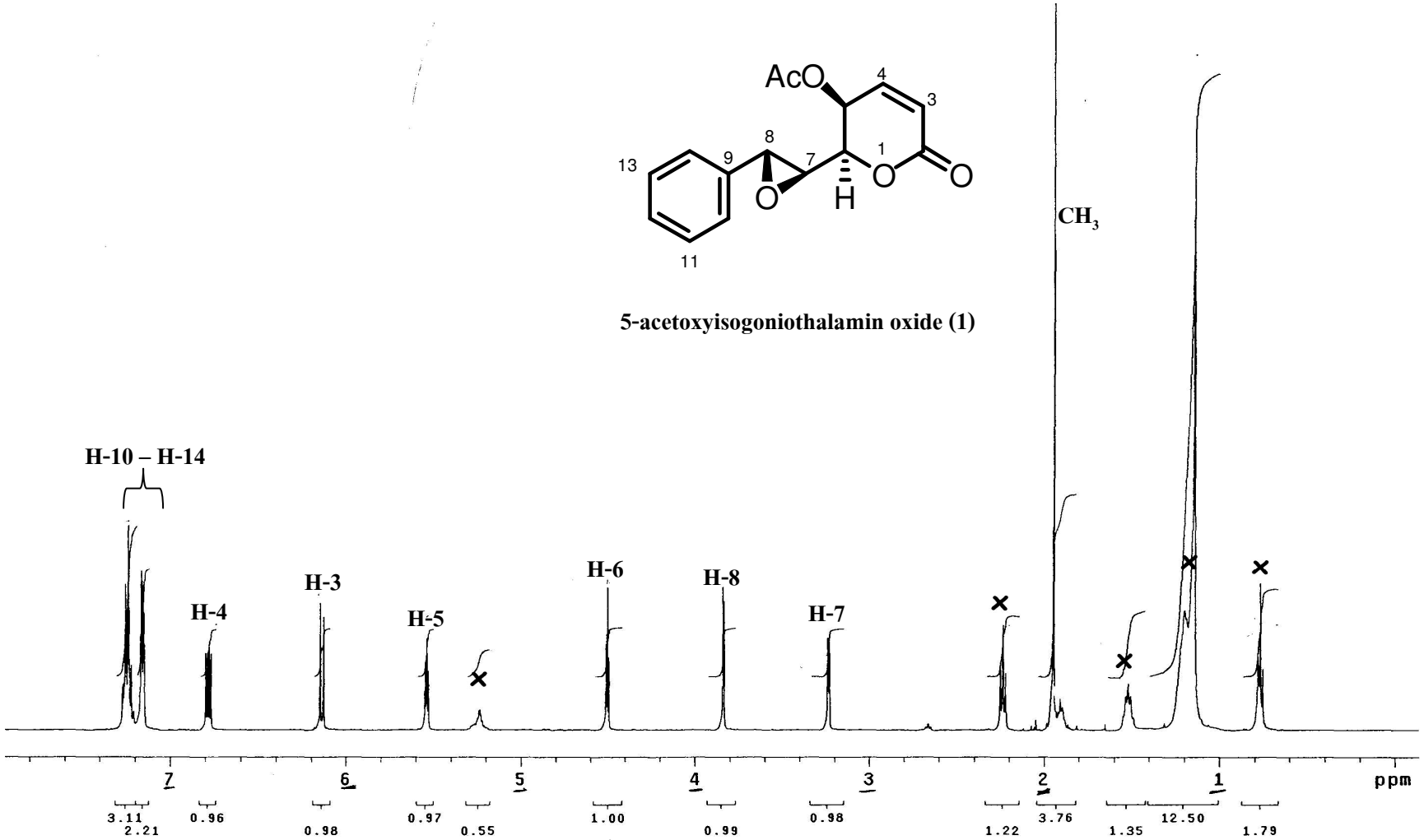


Figure 11: The structure and ^{1}H NMR spectrum of compound 1 (500 MHz in $CDCl_3$)

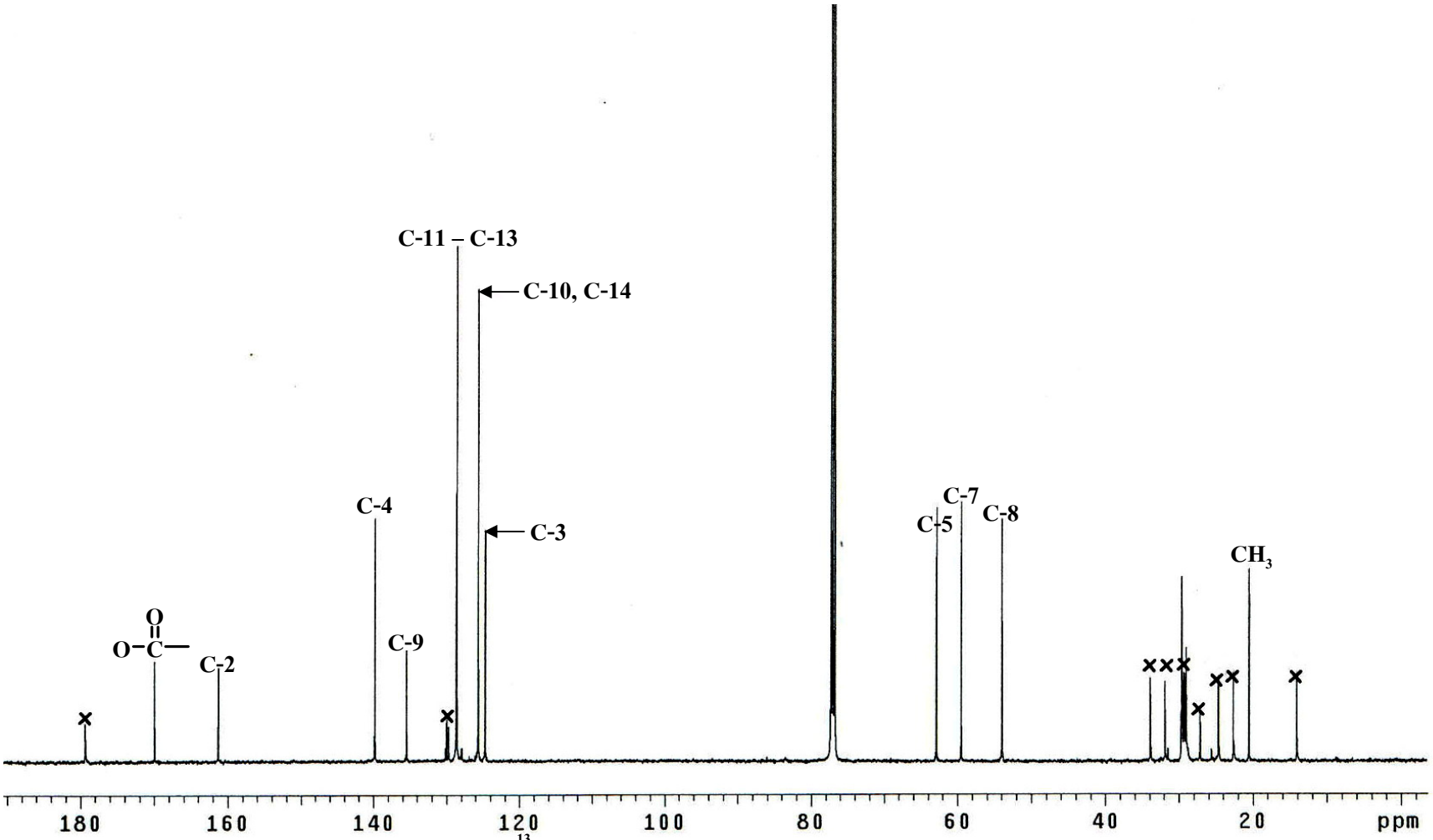


Figure 12: ^{13}C NMR spectrum of compound 1 (125 MHz in $CDCl_3$)

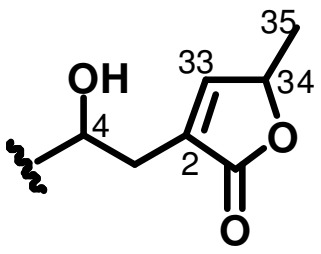
3.3.2 Annonaceous acetogenins (Compounds 4-7)

Compound 4-7 were successively isolated as colorless waxy solids by reversed-phase HPLC and produced very similar ^{13}C and ^{1}H NMR spectra (Tables 10, 11 and 12). The $[M+Na]^+$ peaks of compounds in the low resolution ESIMS at *m/z* 619 suggested their molecular formulas were $C_{35}H_{64}O_7$.

The IR spectra of compounds 4-7 contained absorption peaks at ~3400 cm^{-1} (OH stretching), 2900-2840 cm^{-1} (aliphatic C-H absorption) and ~1730 cm^{-1} (carbonyl group). UV spectrum (in MeOH) showed λ_{max} at 210 nm (compounds 4 and 5) and 212 nm (compounds 6 and 7).

The ^{13}C spectra of each compound showed seven signals of oxygen-bearing carbons in the region δ 69.9- 82.7 while correlated protons resonance in the region δ 3.38-5.06.

Carbonyl group in each compound was established by carbon signal at δ 174.7 (C-1) and confirmed by strong IR absorption (~1730 cm^{-1}). HMBC spectra showed correlations from C-1 to H-3a, H-3b and H-33; C-2 (at δ 131.1) to H-33 and H-34; C-33 (at δ 151.9) to H-3a, H-3b, H-34 and H-35 and C-34 (at δ 78.0) to H-33 and H-35. And the ^{1}H-^{1}H COSY spectra showed correlation between H-34 and H-35, the coupling constant was 7.0 Hz (compound 4) or 6.5 Hz (compounds 5-7). These data represented methylated α,β unsaturated γ-lactone moiety. The correlation of H-3a and H-3b to H-4 (oxygen bearing methine) confirmed the presence of an α,β unsaturated γ-lactone ring with a hydroxyl group at the C-4 position as commonly found among several of the annonaceous acetogenins.



Methylated α,β unsaturated γ-lactone ring with a hydroxyl group at the C-4 position

The spectral data (Figures 13 and 14) of compound 4 showed carbon signals of two methylenes at δ 28.7 (C-17 and C-18) and two oxygen bearing methines at δ 82.6 (C-16) and δ 82.7 (C-19) and proton signals at δ 1.67 (m; H-17a and H-18a), δ 1.99 (m; H-17b and H-18b) and δ 3.80 (q, *J*= 6.5 Hz, H-16 and H-19), represented a mono-THF ring fragment. COSY spectrum showed correlations of oxygen bearing methines, H-15 (at δ 3.41) to H-16 and H-19 to H-20 (at δ 3.41). Therefore, this fragment was flanked with oxygen bearing methines.

The characteristic of an α,β unsaturated γ-lactone ring with a hydroxyl group at the C-4 position and mono-THF ring with two flanking hydroxyls were also shown in spectral data of compound 5-7 (Tables 10, 11 and 12).

The placement of mono-THF ring with two flanking hydroxyls fragment and an oxygen bearing methine of each compound along the aliphatic chain were determined base on the EIMS fragmentation pattern of each compound. The assignments of the peak fragments of compound 4 are presented in Figure 16.

The significant peaks of compound 4 at *m/z* 309 (cleavage between C-15/C-16 – H_2O), 291 (cleavage between C-15/C-16 –$2H_2O$), 273 (cleavage between C-15/C-16 –$3H_2O$), 361 (cleavage between C-19/C-20 –$2H_2O$) and 343 (cleavage between C-19/C-20 –$3H_2O$) allowed placement of the THF-ring at C-16 and C-19. The fragment ions at *m/z* 367 (cleavage between C-9/C-10 –H_2O) and 241 (cleavage between C-10/C-11) suggested that the remaining a methine with OH group at δ 3.59 should be assigned at C-10.

The stereochemistries at C-15/C-16 and C-19/C-20 of compound 4 were concluded to be *threo*, and the stereochemistry of the THF ring was suggested by proton resonances of the two methylene groups in the ring at δ 1.67 and 1.99, as *trans* by comparison with model compounds of known relative configuration (Figure 13), synthesized by Fujimoto et al. (1994).

Therefore, the structure of compound 4 was proposed to be annonacin (Figure 14). This structure was confirmed with the NMR experimental data and the absolute configurations were suggested by comparing the specific rotation from previous report (annonacin: $[\alpha]_D$ = +21 ; c0.51, $CHCl_3$, as reported in Hu et al., 2001). The comparison of NMR data of compound 4 and annonacin was shown in Table 10.

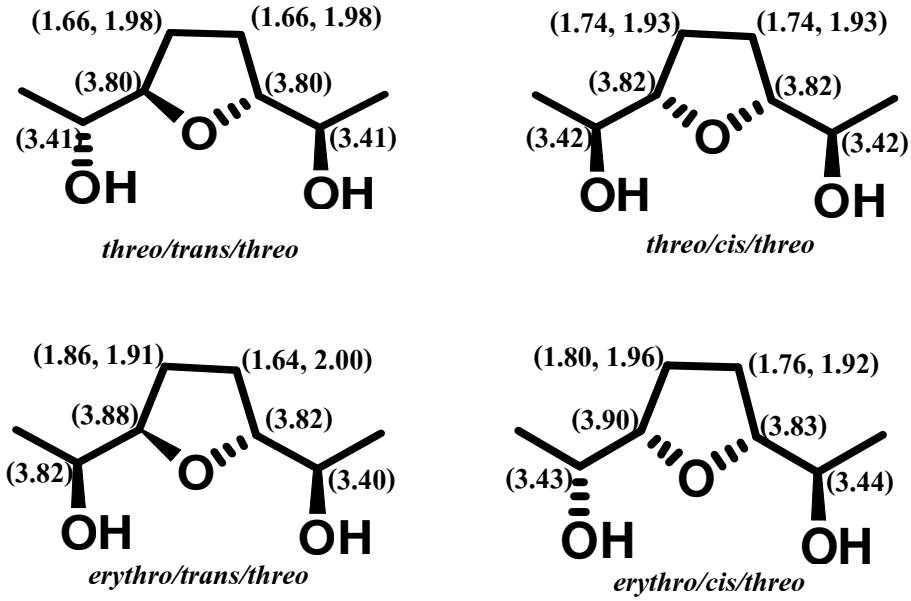


Figure 13: Stereochemistries of tetrahydrofurans (Fujimoto et al., 1994)

The EIMS fragmentation pattern of compound 5 showed in Figure 21, similar to compound 4. While, This compound showed methylene proton signals at δ 1.75 (2H, H-17a and H-18a) and 1.94 (2H, H-17b and H-18b) (Figure 18), different to compound 4 and the slight downfield shift of the ether methylene protons to δ 3.82 (2H, H-16 and H-19) and carbon signals of the methylene groups in the ring were slightly shifted to δ 28.1 indicated that the ring in this compound bore the *threo-cis-threo* configuration (Fujimoto et al., 1994; Rieser et al., 1996 and Liaw et al, 2004). Therefore, compound 5 was proposed to be *cis*-annonacin (Figure 18) which was confirmed base on NMR experimental data and specific rotation from report of Rieser et al. (1996) (*cis*-annonacin: $[\alpha]_D$ = +10; c 0.17, $CHCl_3$).

Table 10: Comparison of NMR data of compound 4 and annonacin

Position	C type	Compound 4 [a]			Annonacin [b] (Hu et al., 2001)		
		$\delta^{13}C$	$\delta^{1}H$	mult. (*J* in Hz)	$\delta^{13}C$	$\delta^{1}H$	mult. (*J* in Hz)
1	C	174.7	-		174.6	-	
2	C	131.1	-		131.2	-	
3	CH_2	33.3	2.40	dd (15.0, 8.5)	33.4	2.40	dd(14.7, 7.8)
			2.52	dt (15.0, 1.5)		2.52	d(14.7)
4	CH	69.9	3.84	unclassified	69.9	3.85	m
5	CH_2	37.2	1.48	unclassified	25.5-37.4	1.20-1.60	m
6-9	CH_2	25.5-37.3	1.25-1.48	unclassified	25.5-37.4	1.20-1.60	m
10	CH	71.7	3.59	m	71.7	3.59	m
11-13	CH_2	25.5-37.3	1.25-1.48	unclassified	25.5-37.4	1.20-1.60	m
14	CH_2	33.3	1.41	unclassified	33.5	1.20-1.60	m
15	CH	74.0	3.41	unclassified	74.0	3.41	dt(11.7, 6.0)
16	CH	82.6	3.80	unclassified	82.6	3.81	dt (11.7, 6.6)
17, 18	CH_2	28.7	1.67	unclassified	28.7	1.68	m
			1.99	unclassified		1.99	m
19	CH	82.7	3.80	unclassified	82.7	3.81	dt(11.7,6.6)
20	CH	74.1	3.41	unclassified	74.1	3.41	dt(11.7, 6.0)
21-30	CH_2	25.5-37.3	1.25-1.48	unclassified	25.5-37.4	1.20-1.60	m
31	CH_2	22.7	1.25	unclassified	22.7	1.20-1.60	m
32	CH_3	14.1	0.88	t (7.0)	14.1	0.88	t(6.8)
33	CH	151.9	7.19	s	151.8	7.18	s
34	CH	78.0	5.06	q (7.0)	78.0	5.06	q (6.6)
35	CH_3	19.1	1.44	d (7.0)	19.1	1.43	d(7.2)

[a] 500 MHz for ^{1}H and 125 MHz for ^{13}C in $CDCl_3$

[b] 600 MHz for ^{1}H and 150 MHz for ^{13}C in $CDCl_3$

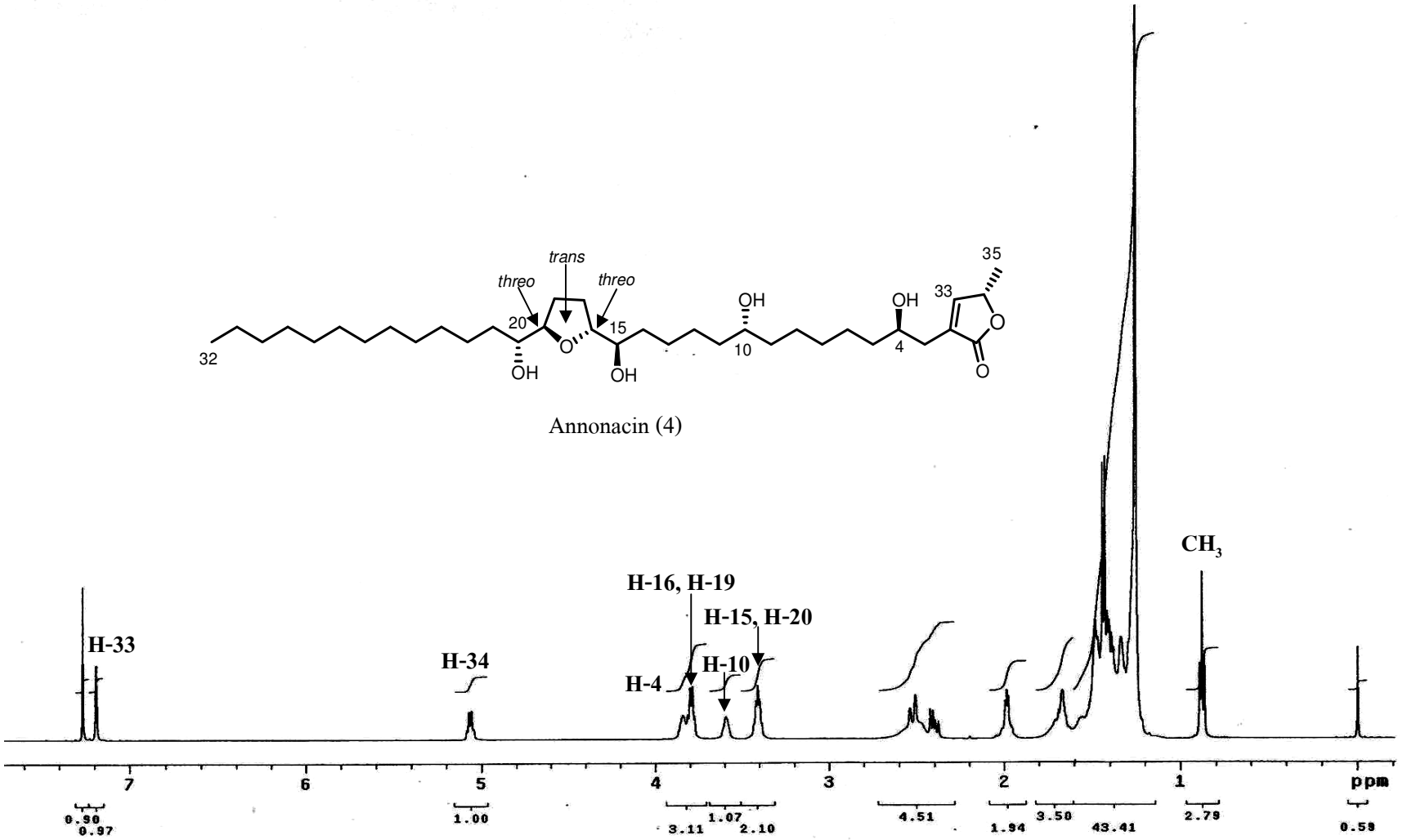


Figure 14: The structure and ^{1}H NMR spectrum of compound 4 (500 MHz in $CDCl_3$)

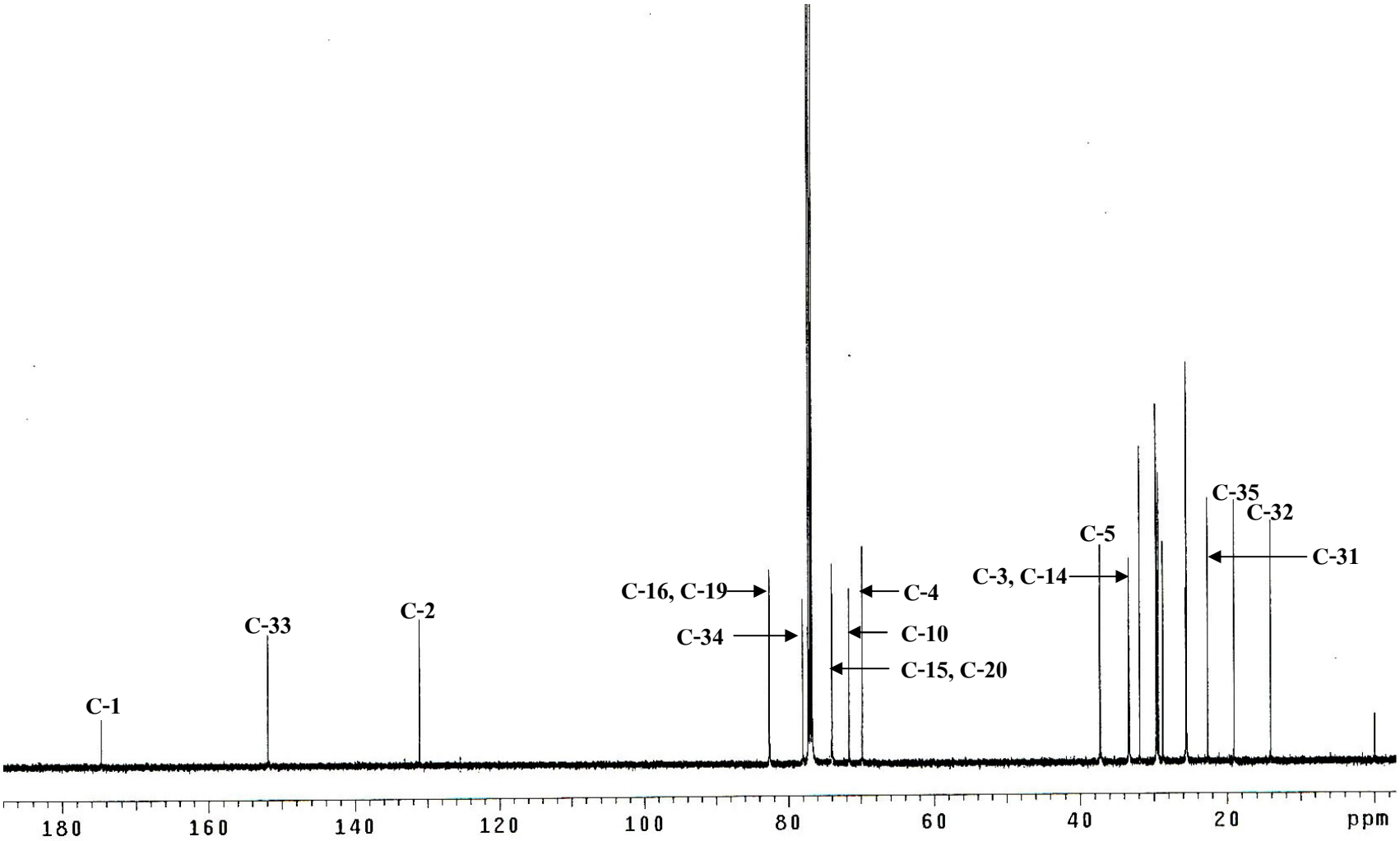


Figure 15: ^{13}C NMR spectrum of compound 4 (125 MHz in $CDCl_3$)

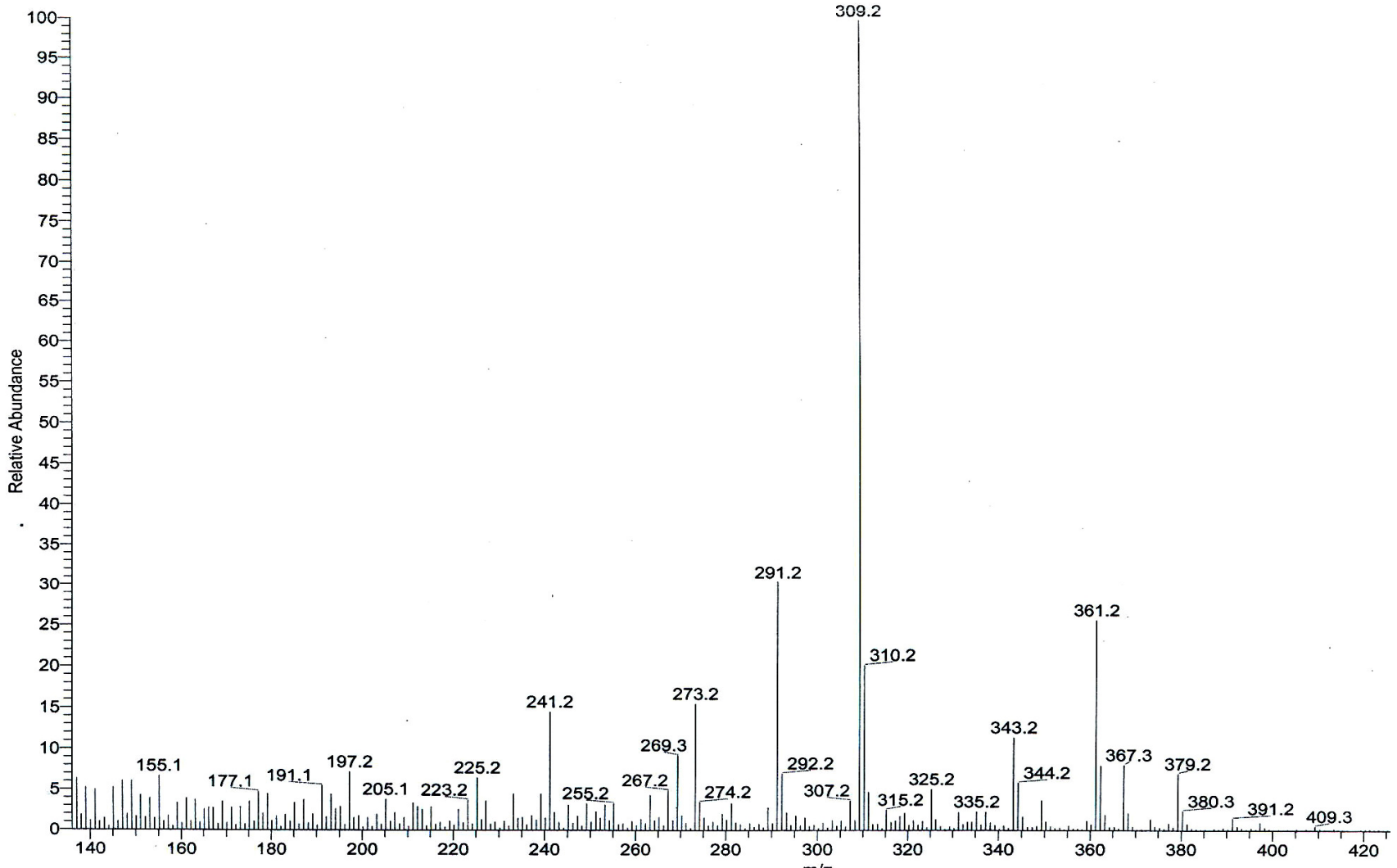


Figure 16: EIMS spectrum of compound 4

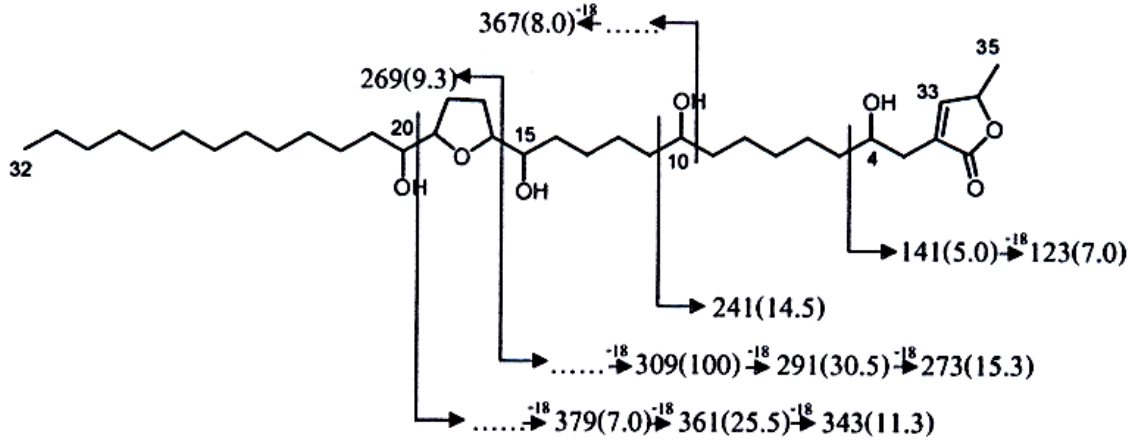


Figure 17: EIMS fragmentation (*m/z*) of compound 4 with percent intensities reported in parentheses; losses of H_2O indicated by 18 *m/z*.

Table 11: NMR data of compound 5 (500 MHz for ^{1}H and 125 MHz for ^{13}C in $CDCl_3$)

Position	C type	Compound 5		
		$\delta^{13}C$	$\delta^{1}H$	mult. (*J* in Hz)
1	C	174.7	-	
2	C	131.1	-	
3	CH_2	33.4	2.40	ddt (15.0, 8.5,1.5)
			2.53	dt (15.0, 1.5)
4	CH	69.9	3.85	unclassified
5	CH_2	37.2	1.48	unclassified
6-9	CH_2	25.4-37.3	1.25-1.48	unclassified
10	CH	71.7	3.60	m
11-13	CH_2	25.4-37.3	1.25-1.48	unclassified
14	CH_2	34.1	1.46	unclassified
15	CH	74.3	3.43	unclassified
16	CH	82.6	3.82	unclassified
17, 18	CH_2	28.1	1.75	unclassified
			1.94	unclassified
19	CH	82.7	3.82	unclassified
20	CH	74.3	3.43	unclassified
21-30	CH_2	25.4-37.3	1.25-1.48	unclassified
31	CH_2	22.7	1.25	unclassified
32	CH_3	14.1	0.88	t (7.0)
33	CH	151.9	7.19	d (1.5)
34	CH	78.0	5.06	qd (6.5, 1.5)
35	CH_3	19.1	1.44	d (6.5)

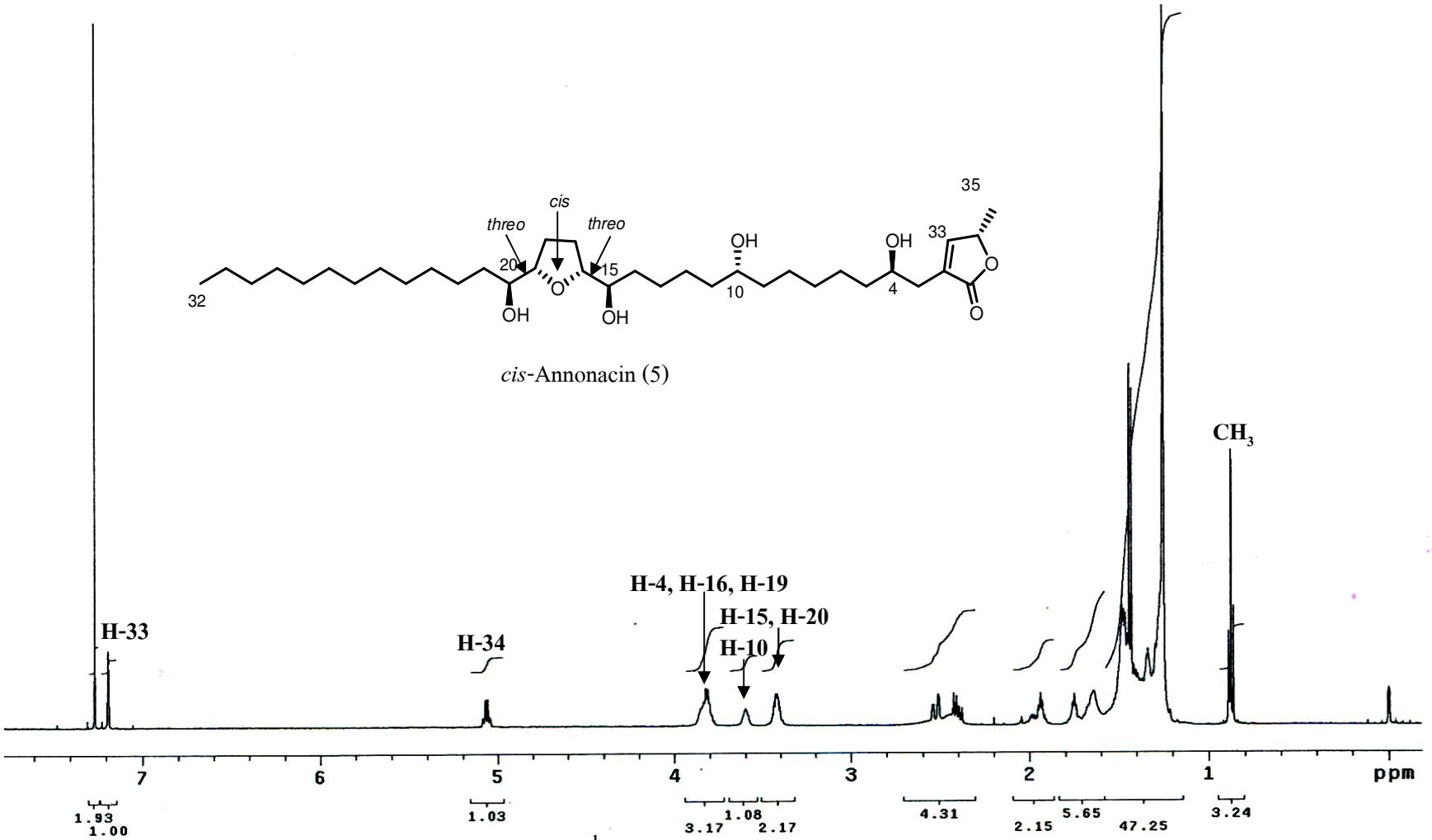


Figure 18: The structure and ^{1}H NMR spectrum of compound 5 (500 MHz in $CDCl_3$)

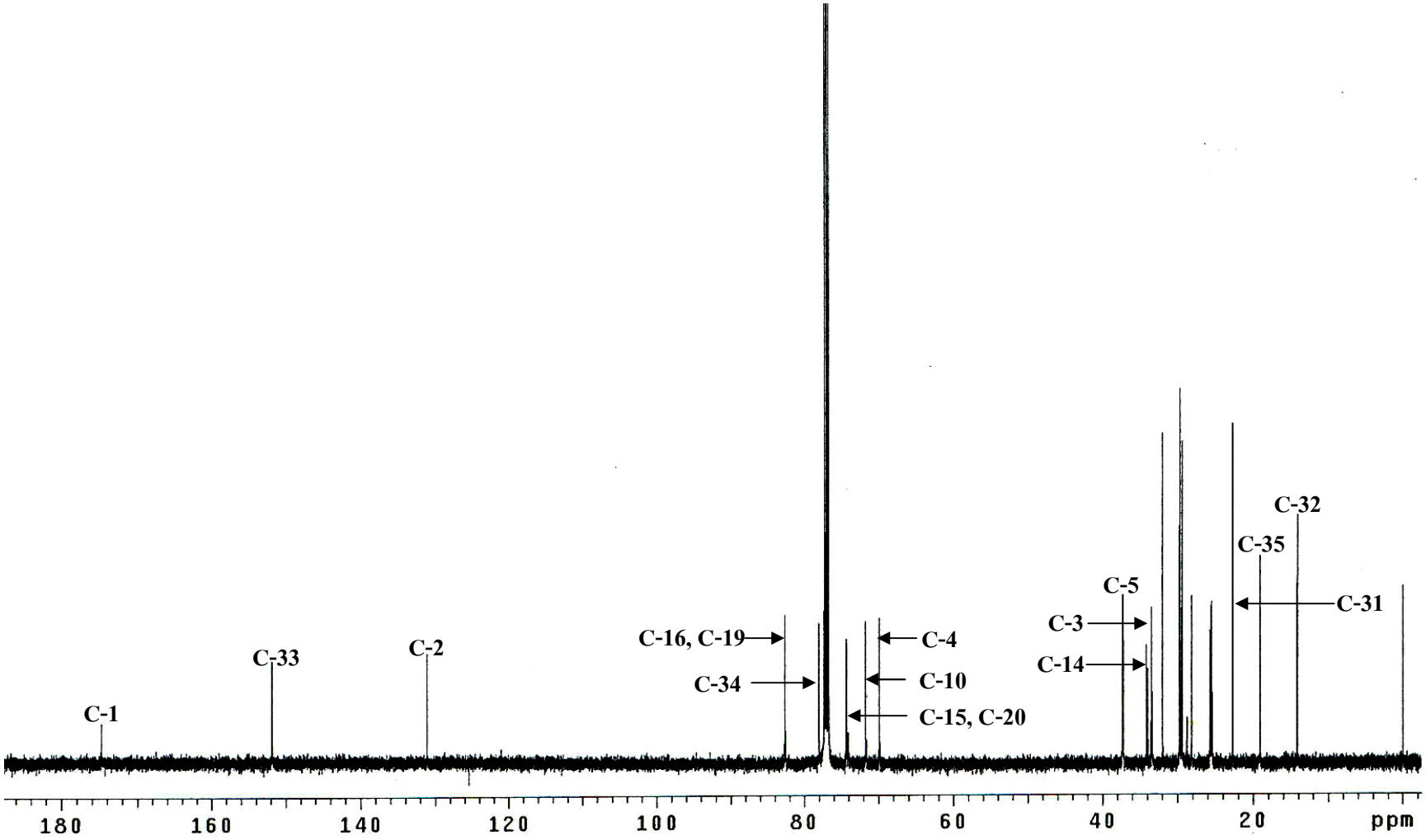


Figure 19: ^{13}C NMR spectrum of compound 5 (125 MHz in $CDCl_3$)

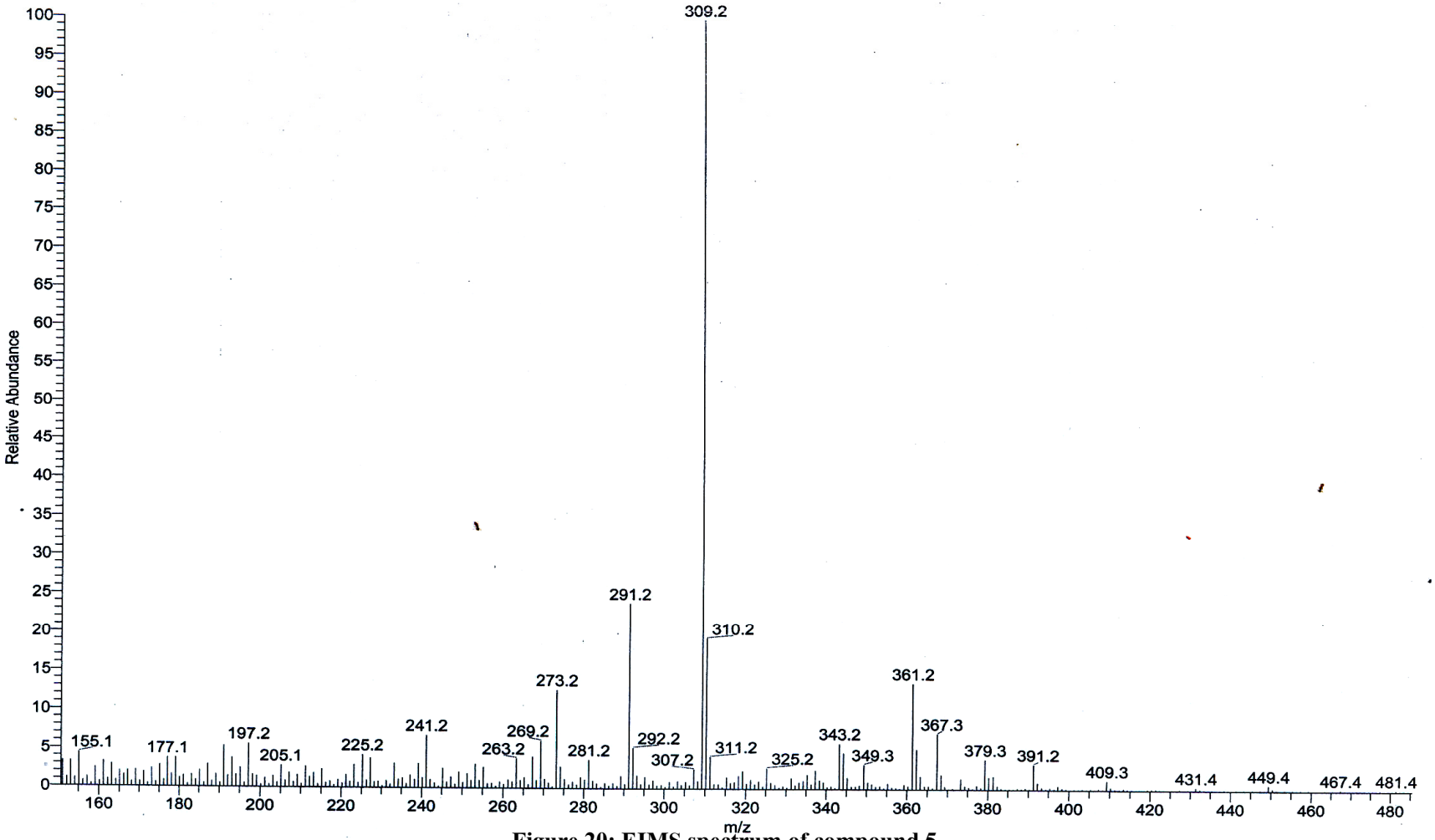


Figure 20: EIMS spectrum of compound 5

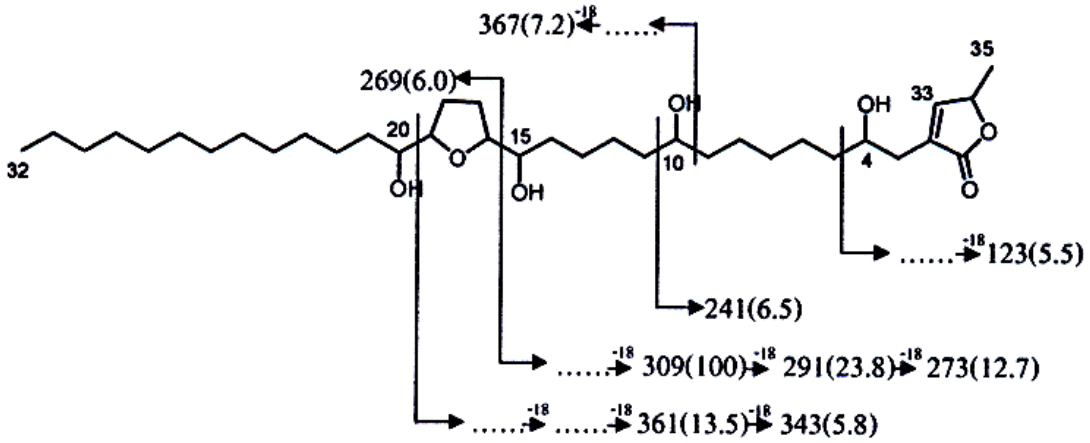
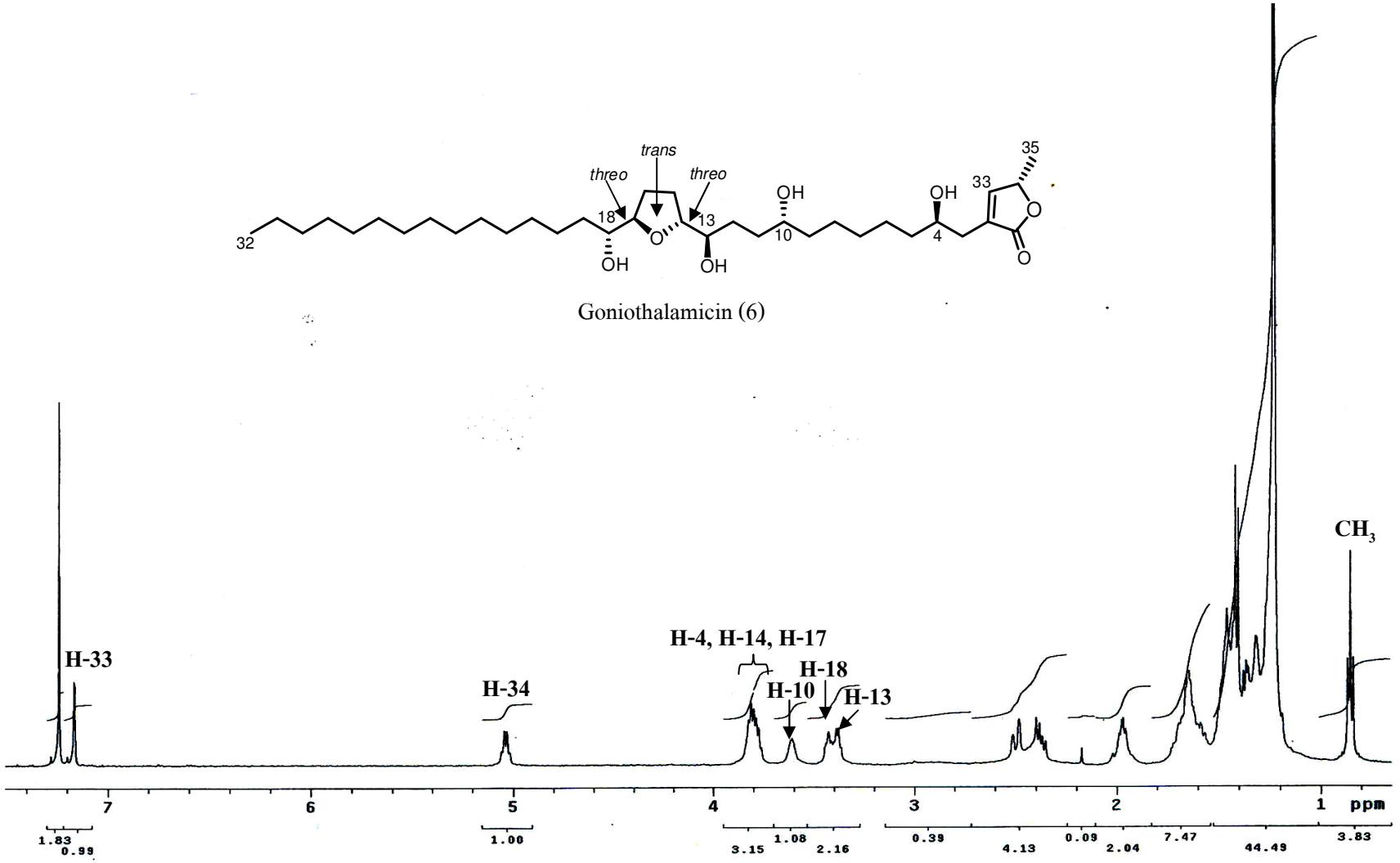


Figure 21: EIMS fragmentation (*m/z*) of compound 5 with percent intensities reported in parentheses; losses of H_2O indicated by 18 *m/z*.

Compound 6 and 4 produced very similar spectral data (Figures 22 and 14). While, The EIMS experiment data showed the THF-ring of compound 6 was placed at C-14 and C-17 based on the EIMS fragments at *m/z* 281 (cleavage between C-13/C-14 $-H_2O$), 351 (cleavage between C-17/C-18 – H_2O) and 333 (cleavage between C-17/C-18 $-2H_2O$) and the oxygen bearing methine was located at C-10 based on the EIMS fragment at *m/z* 241 (cleavage between C-10/C-11) (Figure 25). The *threo-trans-threo* THF ring configuration was also found in compound 6 by indicating proton resonances at δ 1.65 (2H, H-15a and H-16a) and 1.97 (2H, H-15b and H-16b) of methylene groups and at δ 3.38 (H-13), δ 3.43 (H-18), δ 3.77 (H-14), 3.79 (H-17) of oxygen bearing methines. This structure was proposed to be goniothalamicin (Figure 21). The absolute configuration of this compound was suggested by comparing the specific rotation with the previous report (goniothalamicin: $[\alpha]_D$ = +15.5 ; *c*0.23, $CHCl_3$, as reported in Rieser et al., 1996).



Goniothalamicin (6)

Figure 22: The structure and ^{1}H NMR spectrum of compound 6 (500 MHz in $CDCl_3$)

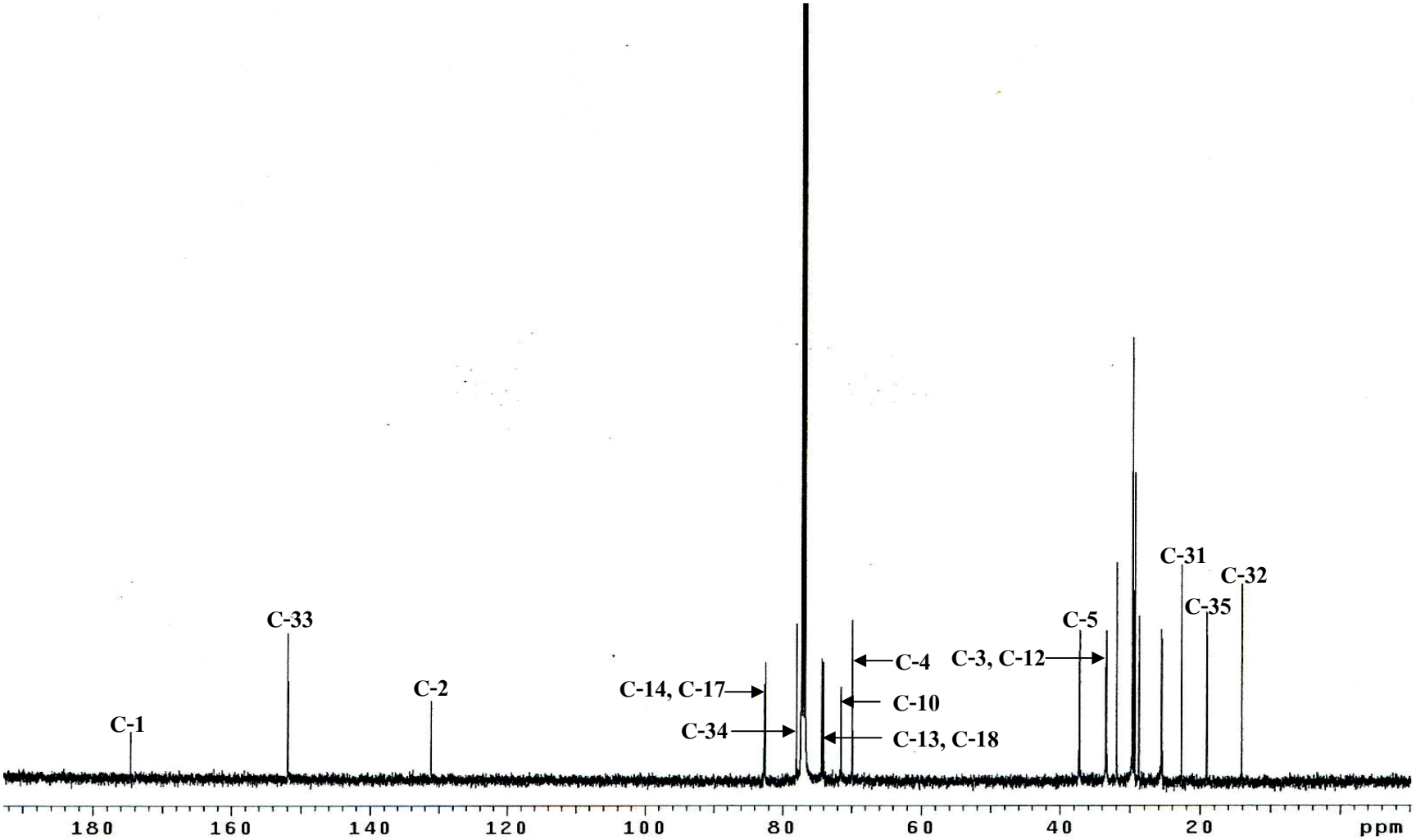


Figure 23: ^{13}C NMR spectrum of compound 6 (125 MHz in $CDCl_3$)

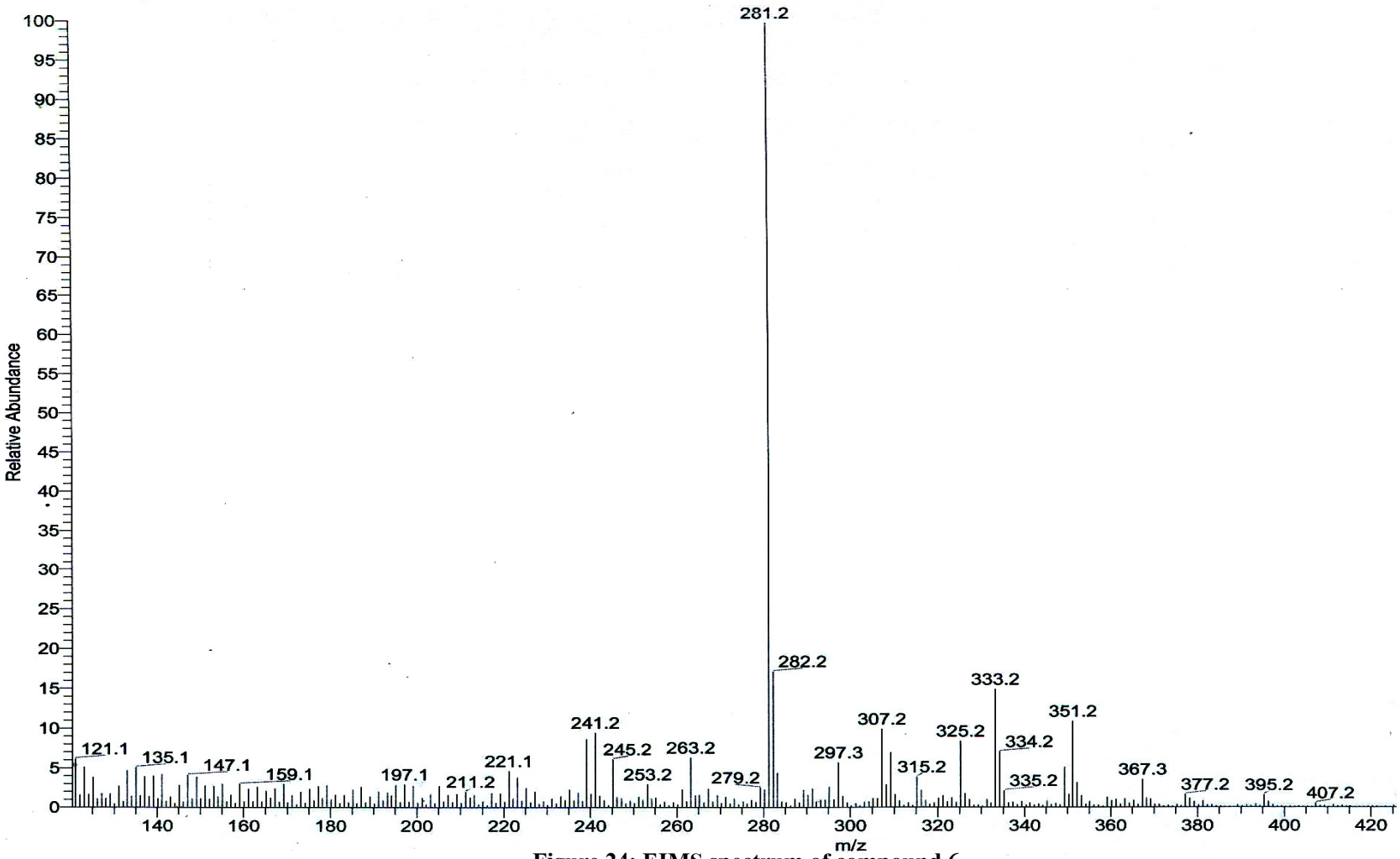


Figure 24: EIMS spectrum of compound 6

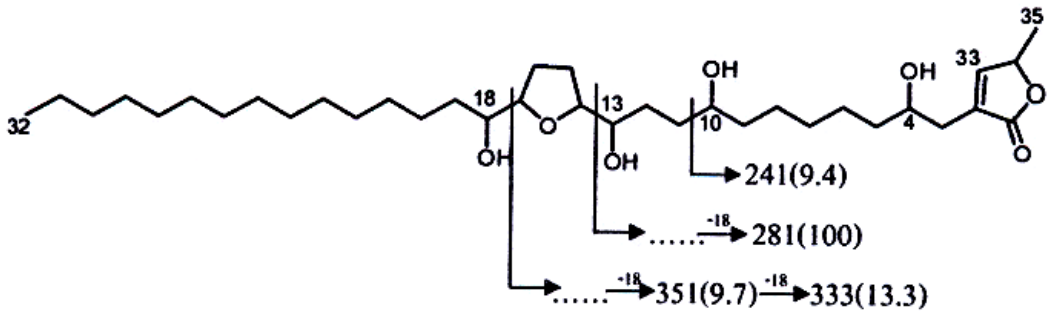


Figure 25: EIMS fragmentation (*m/z*) of compound 6 with percent intensities reported in parentheses; losses of H_2O indicated by 18 *m/z*.

Table 12: NMR data of compound 6 and 7 (500 MHz for ^{1}H and 125 MHz for ^{13}C in $CDCl_3$)

Position	C type	Compound 6			Compound 7		
		$\delta^{13}C$	$\delta^{1}H$	mult. (*J* in Hz)	$\delta^{13}C$	$\delta^{1}H$	mult. (*J* in Hz)
1	C	174.7	-		174.7	-	
2	C	131.1	-		131.1	-	
3	CH_2	33.4	2.38	dd (15.0, 8.0)	33.4	2.38	dd (15.0, 8.5)
			2.50	d (15.0)		2.50	dt (15.0, 1.5)
4	CH	69.9	3.81	unclassified	69.9	3.83	unclassified
5	CH_2	37.2	1.46	unclassified	37.2	1.46	unclassified
6-9	CH_2	25.5-37.3	1.23-1.46	unclassified	25.4-37.2	1.23-1.46	unclassified
10	CH	71.5	3.61	m	71.6	3.63	m
11	CH_2	25.5-37.3	1.23-1.46	unclassified	25.4-37.2	1.23-1.46	unclassified
12	CH_2	33.5	1.36	unclassified	34.2	1.43	unclassified
13	CH	74.1	3.38	unclassified	74.3	3.39	unclassified
14	CH	82.5	3.77	unclassified	82.7	3.80	unclassified
15, 16	CH_2	28.8	1.65	unclassified	28.1	1.74	unclassified
			1.97	unclassified		1.92	unclassified
17	CH	82.7	3.79	unclassified	82.7	3.80	unclassified
18	CH	74.3	3.43	unclassified	74.5	3.44	unclassified
19-30	CH_2	25.5-37.3	1.23-1.46	unclassified	25.4-37.2	1.23-1.46	unclassified
31	CH_2	22.7	1.23	unclassified	22.7	1.23	unclassified
32	CH_3	14.1	0.86	t (7.0)	14.1	0.86	t (6.5)
33	CH	151.9	7.17	m	151.9	7.16	d (1.5)
34	CH	78.0	5.04	q (6.5)	78.0	5.04	qd (6.5, 1.5)
35	CH_3	19.1	1.41	d (6.5)	19.1	1.41	d (6.5)

The EIMS fragmentation of compound 7 was showed in Figure 28, which was similar to compound 6 (Figure 25). Compound 7 showed proton signals at δ 1.74 (2H, H-15a and H-16a), δ 1.92 (2H, H-15b and 16b), δ 3.39 (H-13) δ 3.44 (H-18) and δ 3.80 (2H, H-14 and H-17) (Figure 26) indicating the stereochemistries at the mono-THF ring in this compound to be *threo-cis-threo* configuration. Therefore, this compound was proposed to be *cis*-goniothalamicin

(Figure 26) and confirmed base on spectral data and optical rotation from the previous report (*cis*-goniothalamicin: $[\alpha]_D$ = +7.2; *c* 0.03, $CHCl_3$,as reported in Rieser et al., 1996).

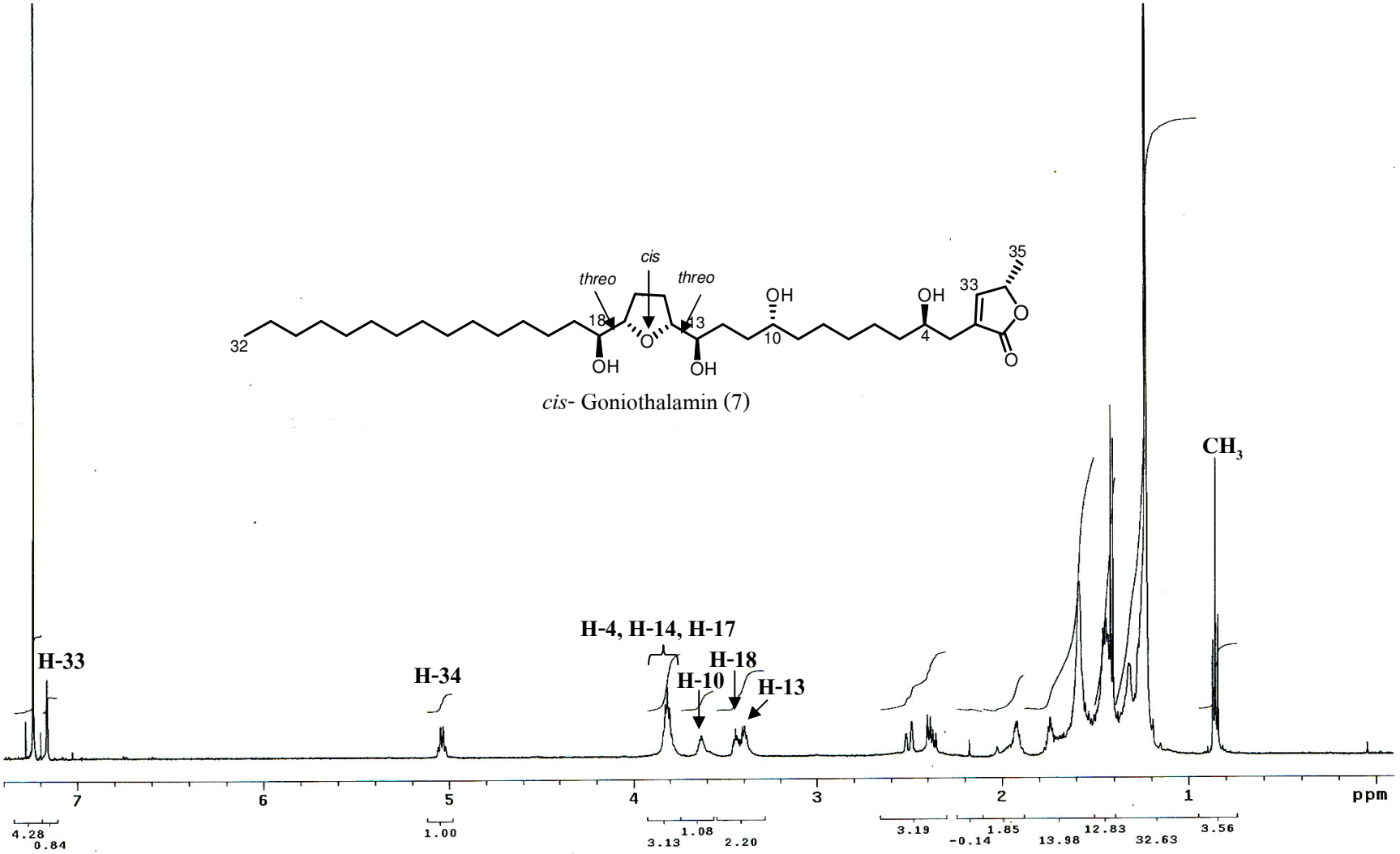


Figure 26: The structure and ^{1}H NMR spectrum of compound 7 (500 MHz in $CDCl_3$)

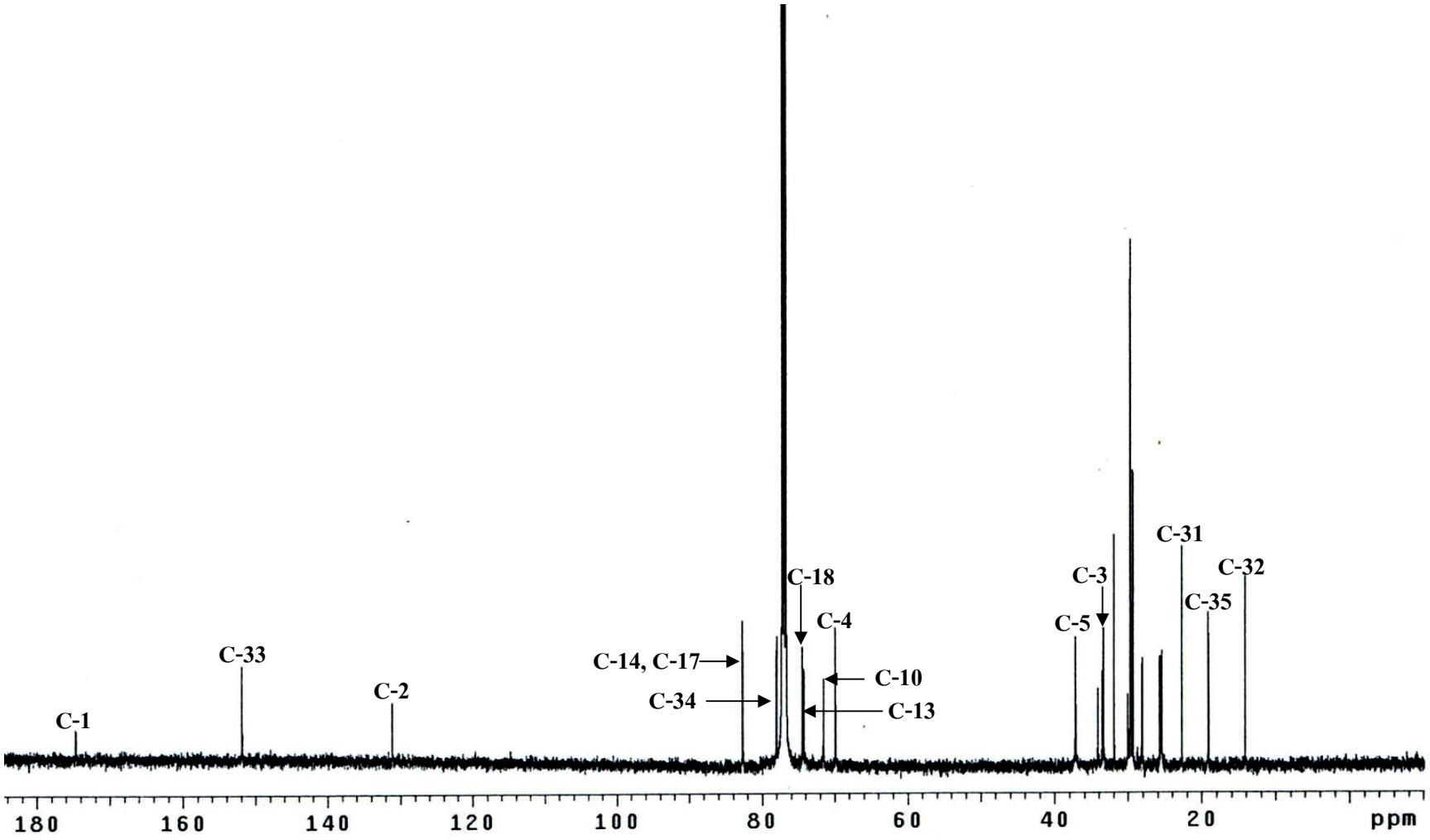


Figure 27: ^{13}C NMR spectrum of compound 7 (125 MHz in $CDCl_3$)

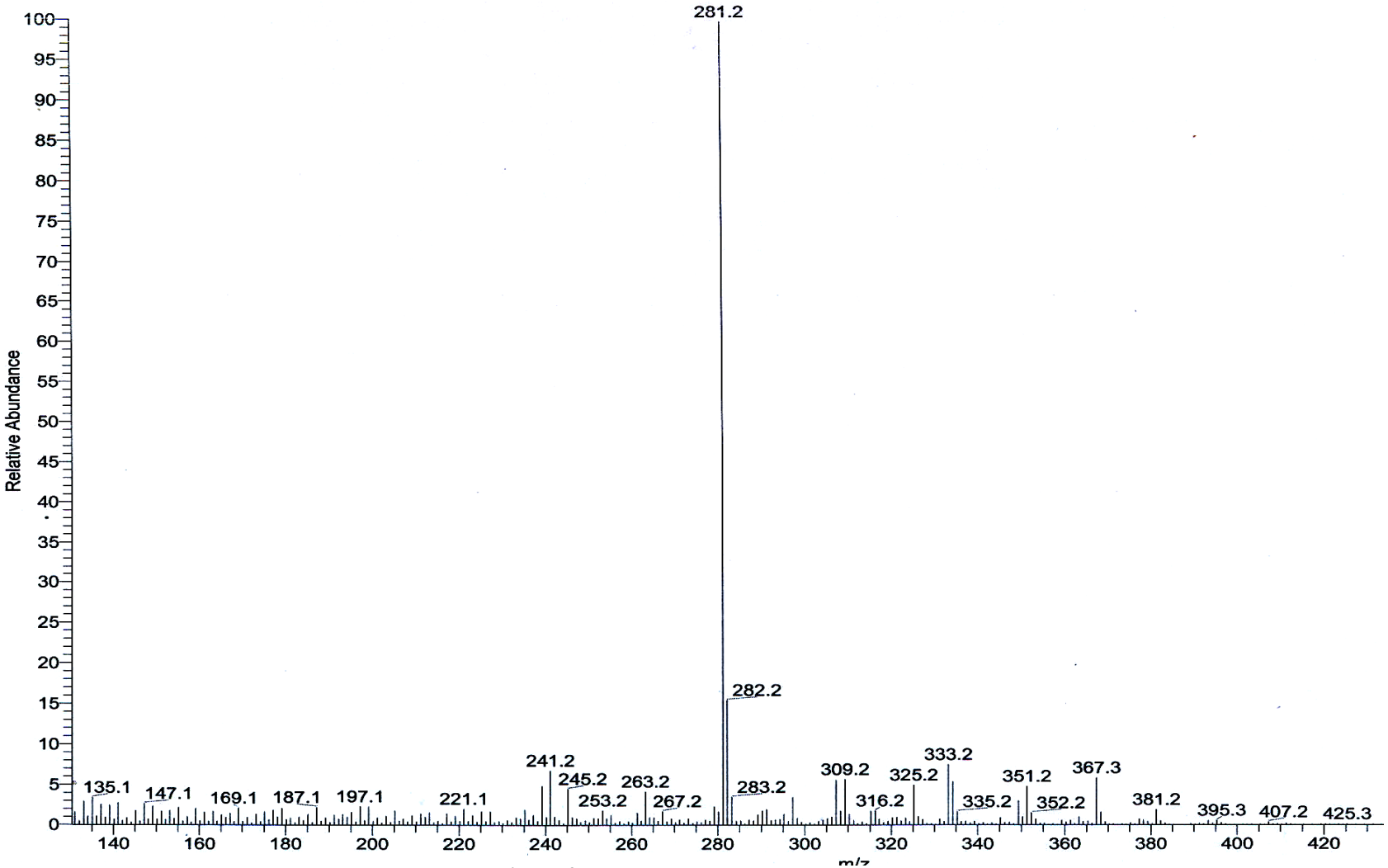


Figure 28: EIMS spectrum of compound 7

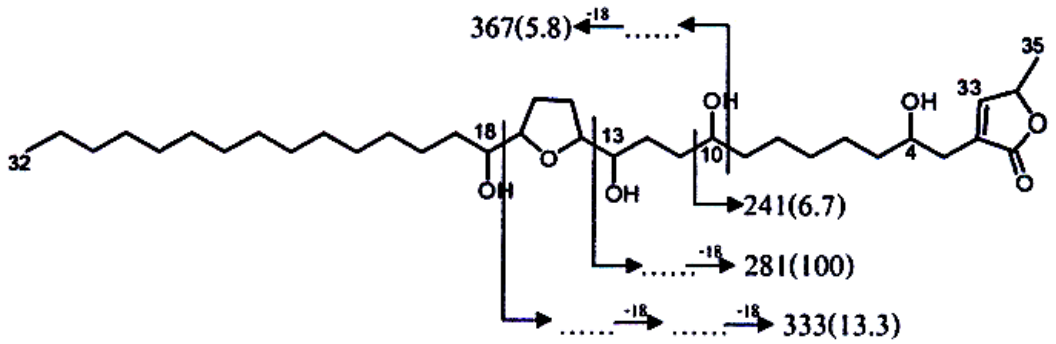


Figure 29: EIMS fragmentation (*m/z*) of compound 7 with percent intensities reported in parentheses; losses of H_2O indicated by 18 *m/z*.

3.4 Cytotoxic activity of isolated compounds

Three styryl lactones and four annonaceous acetogennins were subjected to SRB cytoxicity assay against three human cancer cell lines, COR-L23, MCF-7 and HepG2 and one type of human normal cell line, MRC-5. The results were showed in Table 13.

Table 13: Cytotoxicity of isolated compounds from *G. undulatus* extracts (IC_{50} (µM) ± S.E.M.)

Compound	IC_{50} (µM) ± S.E.M.			
	COR-L23	**MCF-7**	**HepG2**	**MRC-5**
Styryl lactone				
5-Acetoxyisogoniothalamin oxide (1)	7.37±0.20	25.72±1.72	33.89±0.88	48.67±0.20
O-Acetylaltholactone (2)	11.44±0.22	30.40±1.53	77.58±4.57	92.34±0.02
Altholactone (3)	15.43±0.47	59.15±2.11	34.78±1.39	102.82±0.92
Annonaceous acetogenins				
Annonacin (4)	0.56±0.02	2.73±0.13	< 0.17	15.62±0.26
cis-Annonacin (5)	0.54±0.00	2.14±0.19	< 0.17	11.82±0.15
Goniothalamicin (6)	1.68±0.09	2.46±0.13	1.04±0.01	18.38±0.02
cis-Goniothalamicin (7)	1.71±0.27	1.57±0.22	1.01±0.00	31.44±0.08
Vincristine	0.12±0.00	< 0.11	< 0.11	3.51±0.21

These results indicated that all of isolated compounds showed evident cytotoxic activity against COR-L23, MCF-7 and HepG2. Annonaceous acetogenins showed higher cytotoxic activity against all types of cancer cell lines than styryl lactones with IC_{50} values in range of < 0.17-2.46 µM and 7.37-77.58 µM, respectively. However annonaceous acetogenins also have higher cytotoxic activity against normal cell line than styryl lactones with IC_{50} values in range of 11.82-31.44 µM and 48.67-102.82 µM, respectively. Seven compounds exhibited the

less cytotoxicity against normal cell line than vincristine which was used as positive control with IC_{50} in the range of 11.82-102.82 μM.

All of isolated compounds showed more selective activity against cancer cell lines than normal cell line (MRC-5) (Figure 30).

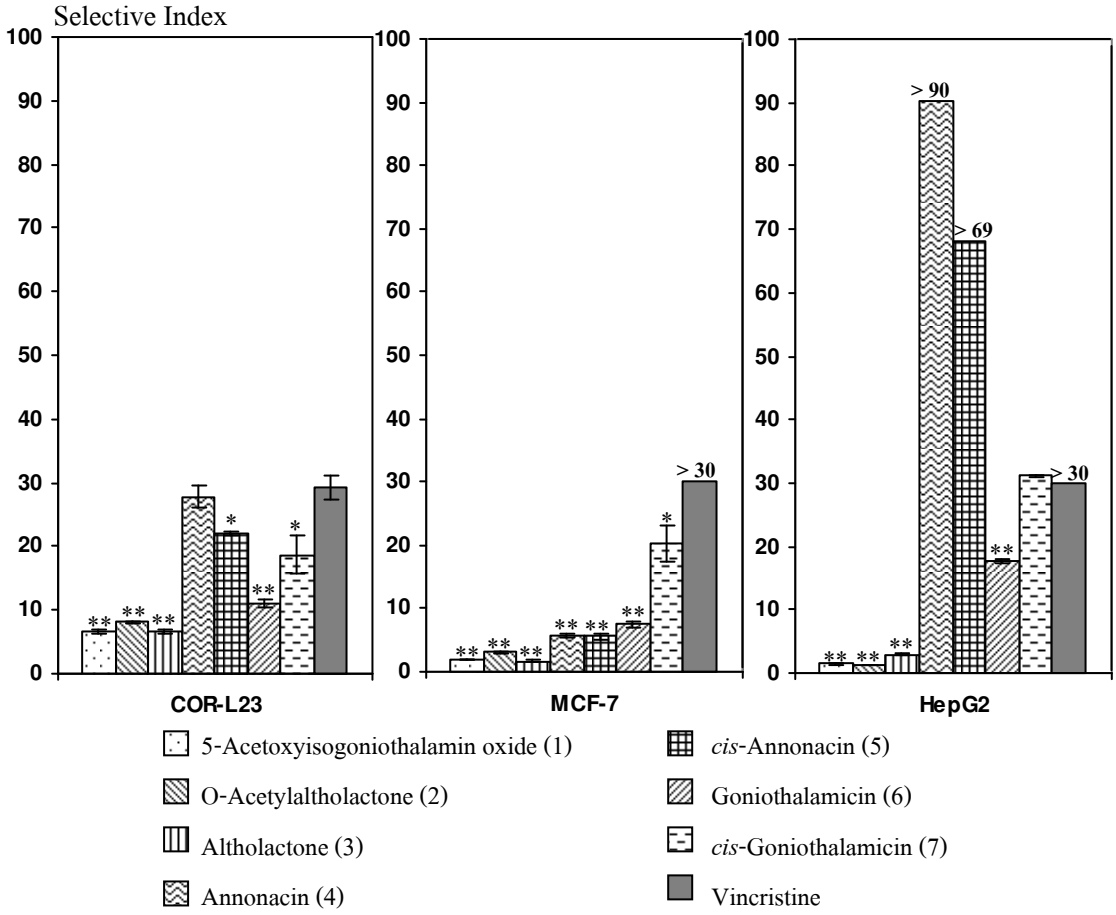


Figure 30: Selectivity Index of isolated compounds (IC_{50} MRC-5/IC_{50} cancer cell) and used Student's *t*-test to compare significant difference between isolated compounds and vincristine. *$P < 0.05$ and **$P < 0.001$.

Annonacin showed more selective cytotoxicity against COR-L23 than MRC-5. The selective index of this compound was 28, not significantly difference when compared with vincristine. Moreover, this compound also showed the highest selective activity against HepG2. The selective index was >90 which may be higher than vincristine about three times.

Peris et al. (2000) have reported that altholactone, O-acetylaltholactone and 5-acetoxyisogoniothalamin oxide were isolated from stem bark of *Goniothalamus arvensis*. These

compounds inhibit the mitochondrial respiratory chain by studying the NADH oxidase activity of beef heart submitochondrial particles. Inhibition of mitochondrial respiratory chain complex I could affect the electron flow through other complexes leading to release of cytochrome c in apoptosis pathway (Inayat-Hussain et al., 2002). The structure activity relationship studies of styryl lactones indicated that the 3, 4-double bond on the lactone ring is the more crucial structural requirement to increase the cytotoxicity (Mereyala and Joe, 2001).

While annonacin, a cytotoxic acetogenin containing a mono-THF ring with two flanking hydroxyls, has been isolated from some genera including *Annona*, *Asimina*, *Goniothalamus*, *Rollinia*, *Uvaria* and *Xylopia*. This compound can activates p21 and arrested cancer cells at a growth-static G1 phase (Yuan et al., 2003) and promotes dopaminergic neuronal death by impairment of energy metabolism (Lannuzel et al., 2003).

cis-Annonacin, goniothalamicin and *cis*-goniothalamicin have been isolated from seeds of *Annona muricata*. All compounds showed activity against the human solid tumor cells including human lung carcinoma cell line (A-549), human breast carcinoma cell line (MCF-7) and human colon adenocarcinoma cell line (HT-29). The IC_{50} were $1x10^{-8}$ – 1.18 µg/ml (Rieser et al., 1996).

The mode of action of acetogenin compounds mainly targets the mitochondrial NADH-ubiquinone oxidoreductase, also known as respiratory complex I of mitochondria complex I which transfers electrons from NADH to ubiquinone and links this process with the translocation of protons across membrane to generate an electrochemical gradient that drives the ATP synthesis (Tormo et al., 1999). The structure activity relationship studies showed that bis-adjacent THF-containing acetogenins are pharmaceutically most potent follwed by mono-THF acetogenins. The lactone moiety seems crucial for activity. Acetogenins with a C35 carbon chain are more active than a C37 carbon chain and a 13-carbon spacer between the γ-lactone ring and the first THF moiety is the most beneficial (Orru et al., 20003).

Therefore, the study of chemical constituents of *G. undulatus* leads to the discovery of cytotoxic agents from natural product which can be developed to useful anticancer drug candidates.

CHAPTER 4

CONCLUSION

In investigation of the cytotoxic agents from *G. undulatus*, seven active compounds were isolated from the root extract including three styryl lactones (5-acetoxyisogoniothalamin oxide (1), O-acetylaltholactone (2) and altholactone (3)) and four annonaceous acetogenins (annonacin (4), *cis*-annonacin (5), goniothalamicin (6) and *cis*-goniothalamicin (7)).

This is the first report about chemical constituents from *G. undulatus*. The isolated acetogenins and styryl lactones were subjected to SRB cytotoxic assay against three types of human cancer cell lines (COR-L23, MCF-7 and HepG2) and one type of human normal cell line (MRC-5). Acetogenins showed higher cytotoxic activity against all of cell types than styryl lactones.

Goniothalamus have been a source of clinically useful compounds, leads for synthetic modification and tools for mechanistic studies. Styryl lactones and acetogenins are attractive for medicinal chemists to study structure-activity relationships leading to a chemotherapeutic agent for treatment of cancer.

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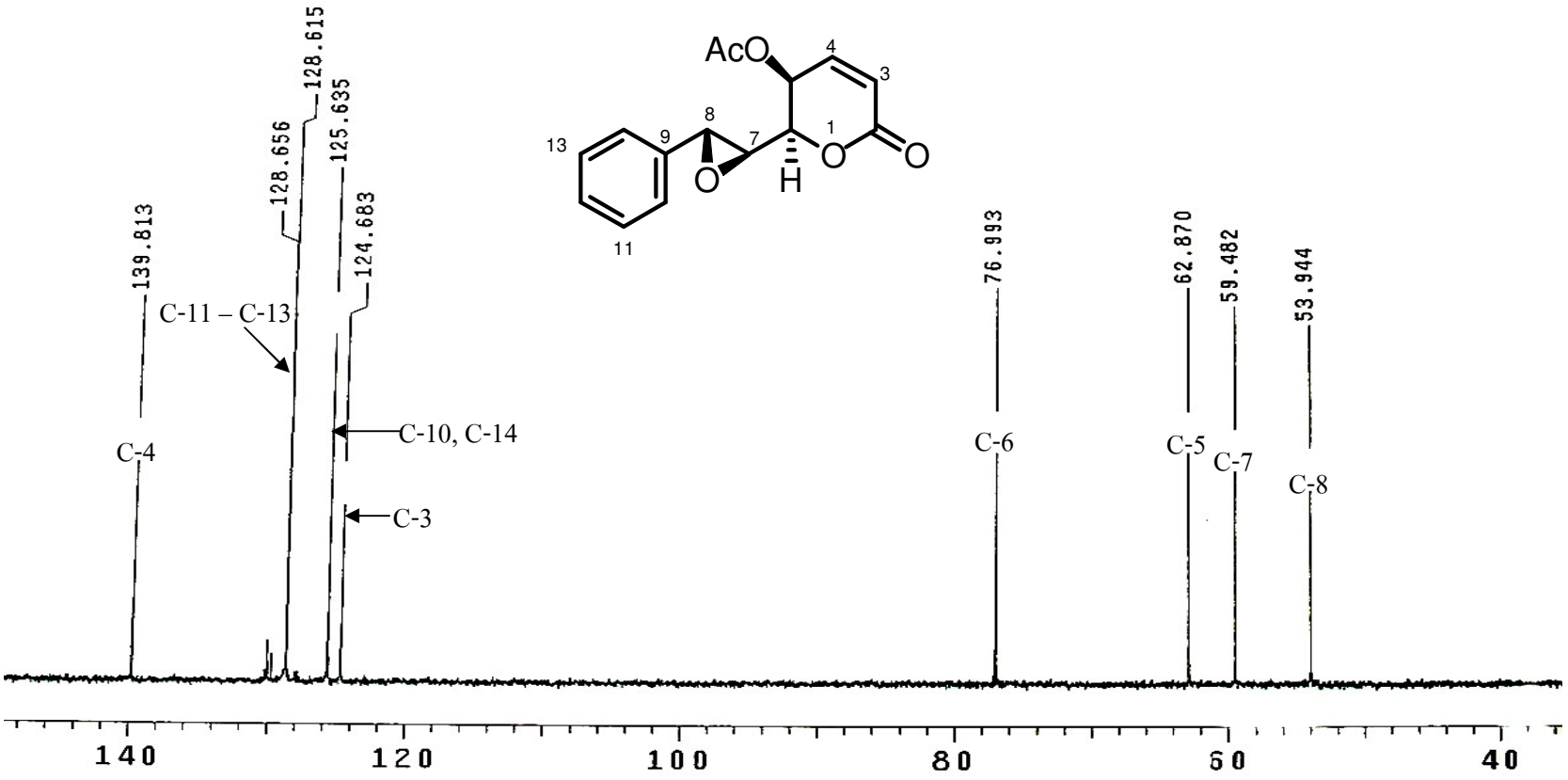
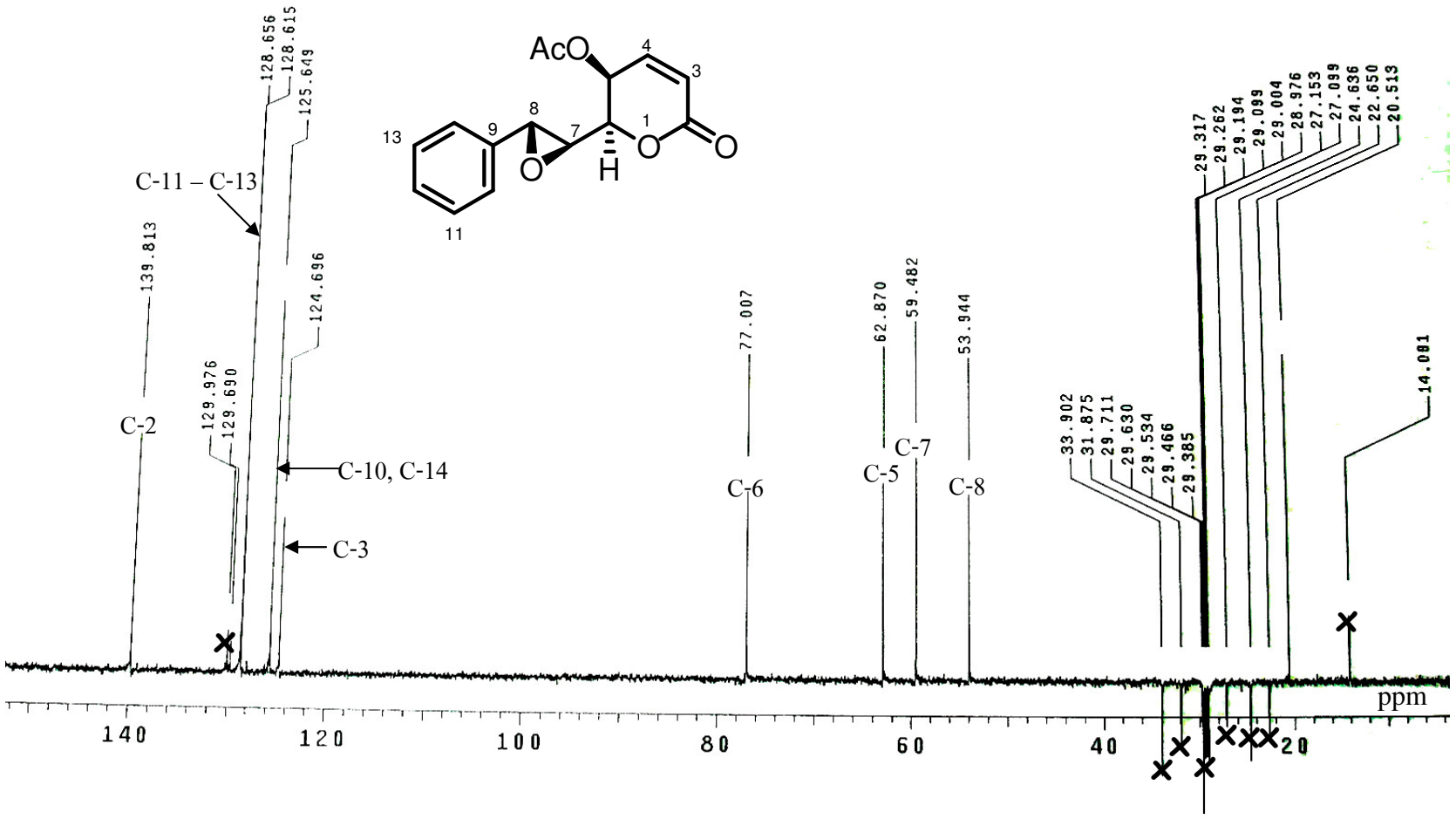
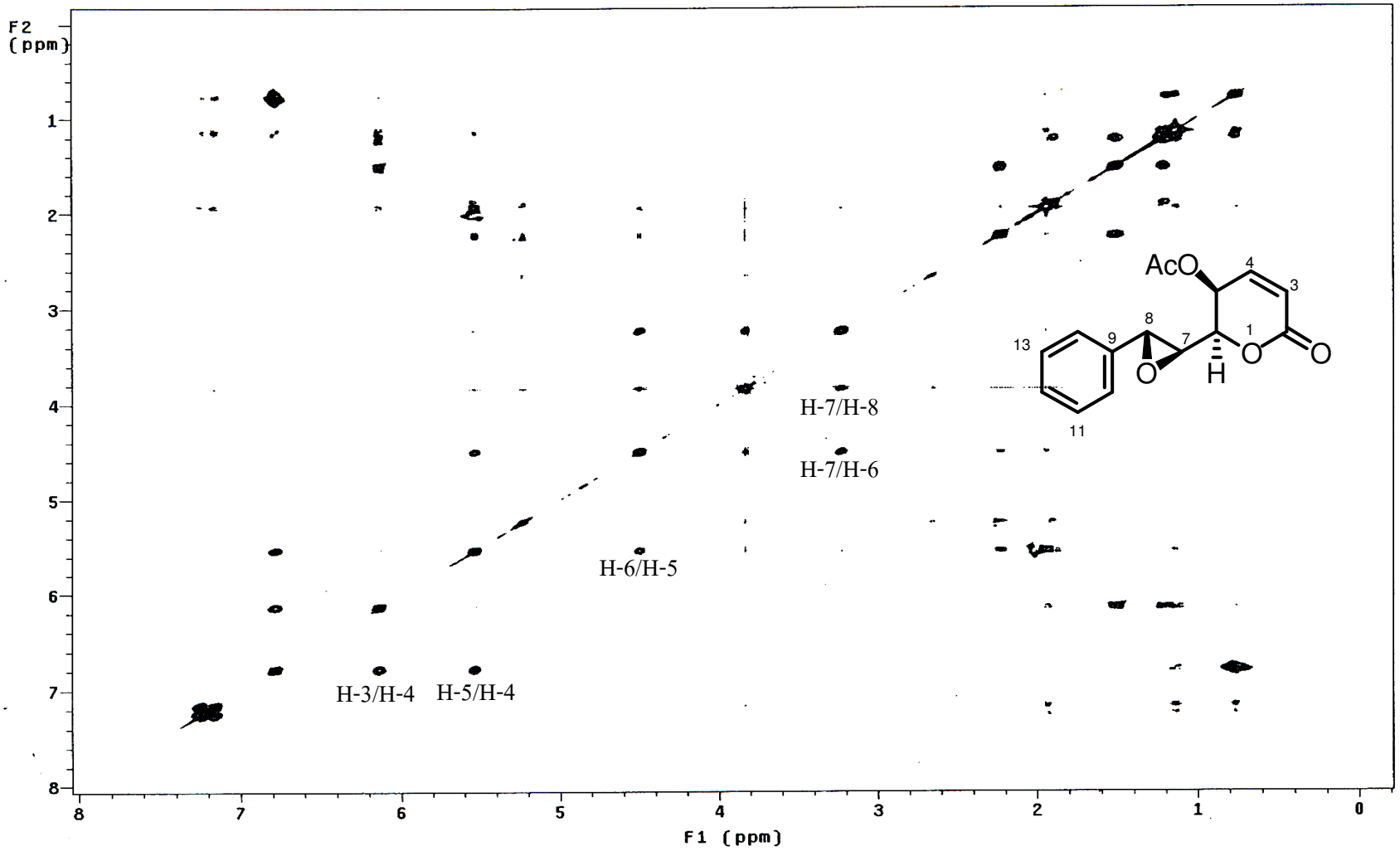
APPENDIX



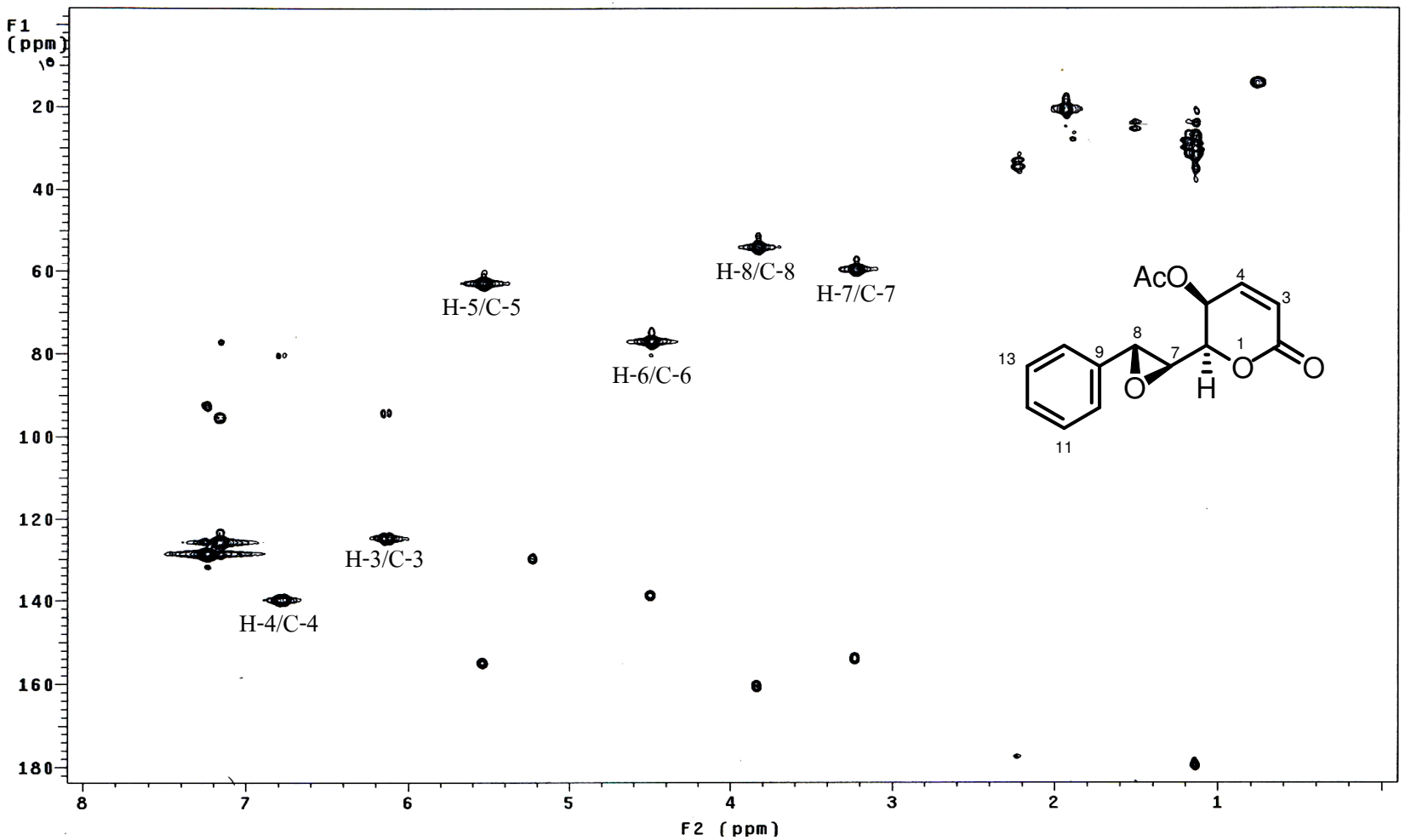
DEPT (90) spectrum of compound 1 in $CDCl_3$



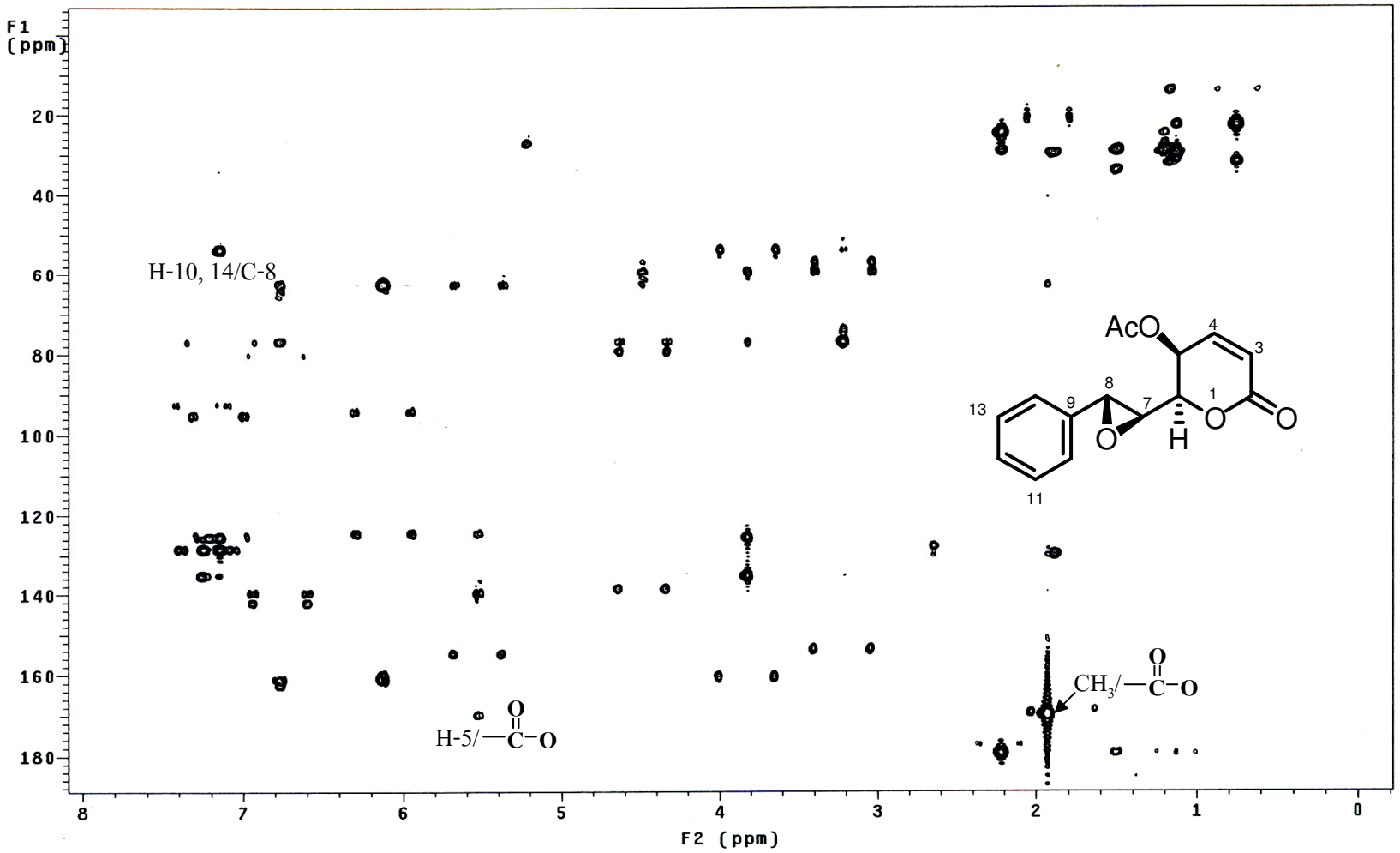
DEPT (135) spectrum of compound 1 in $CDCl_3$



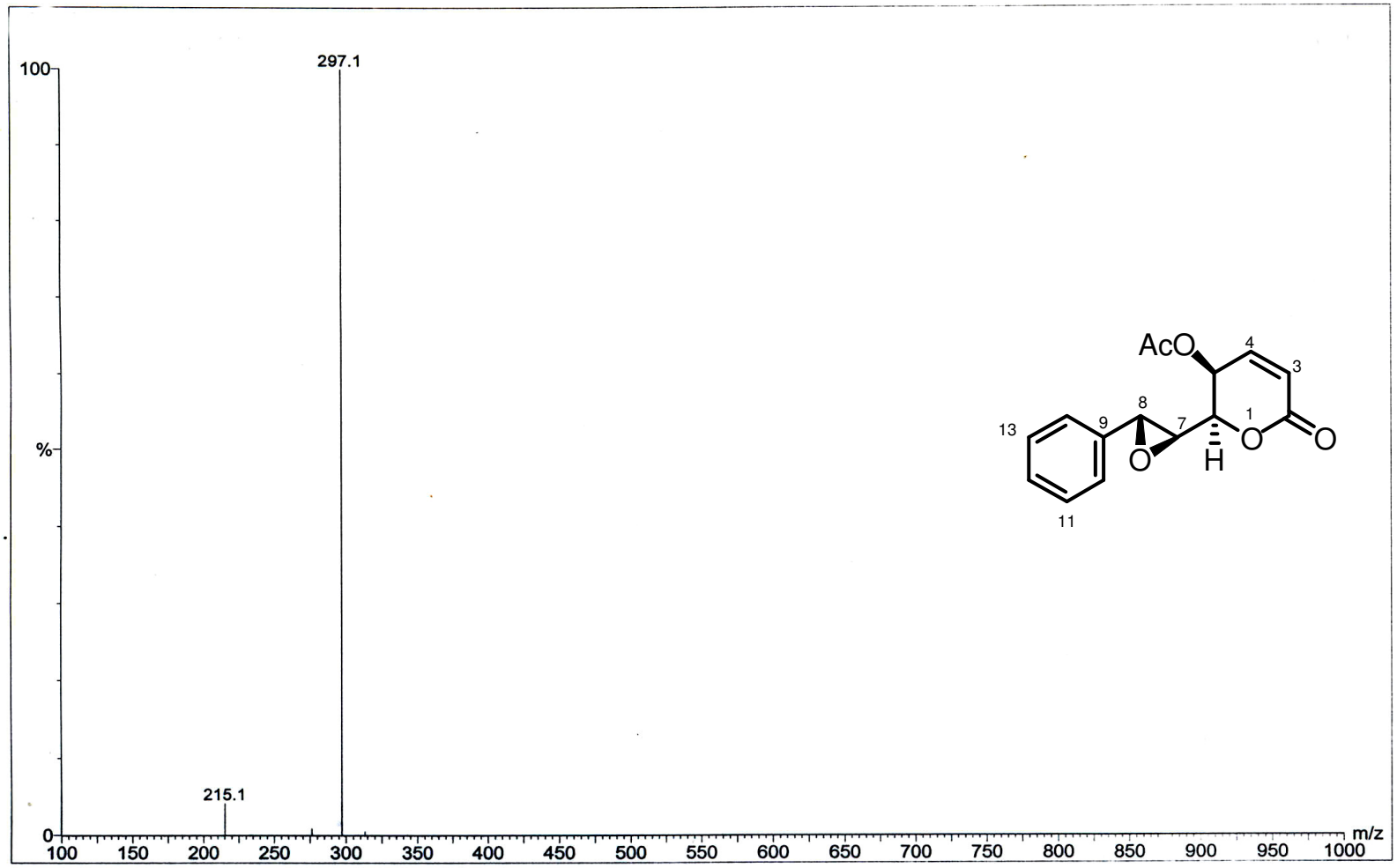
^{1}H-^{1}H COSY spectrum of compound 1 (500 MHz, $CDCl_3$)



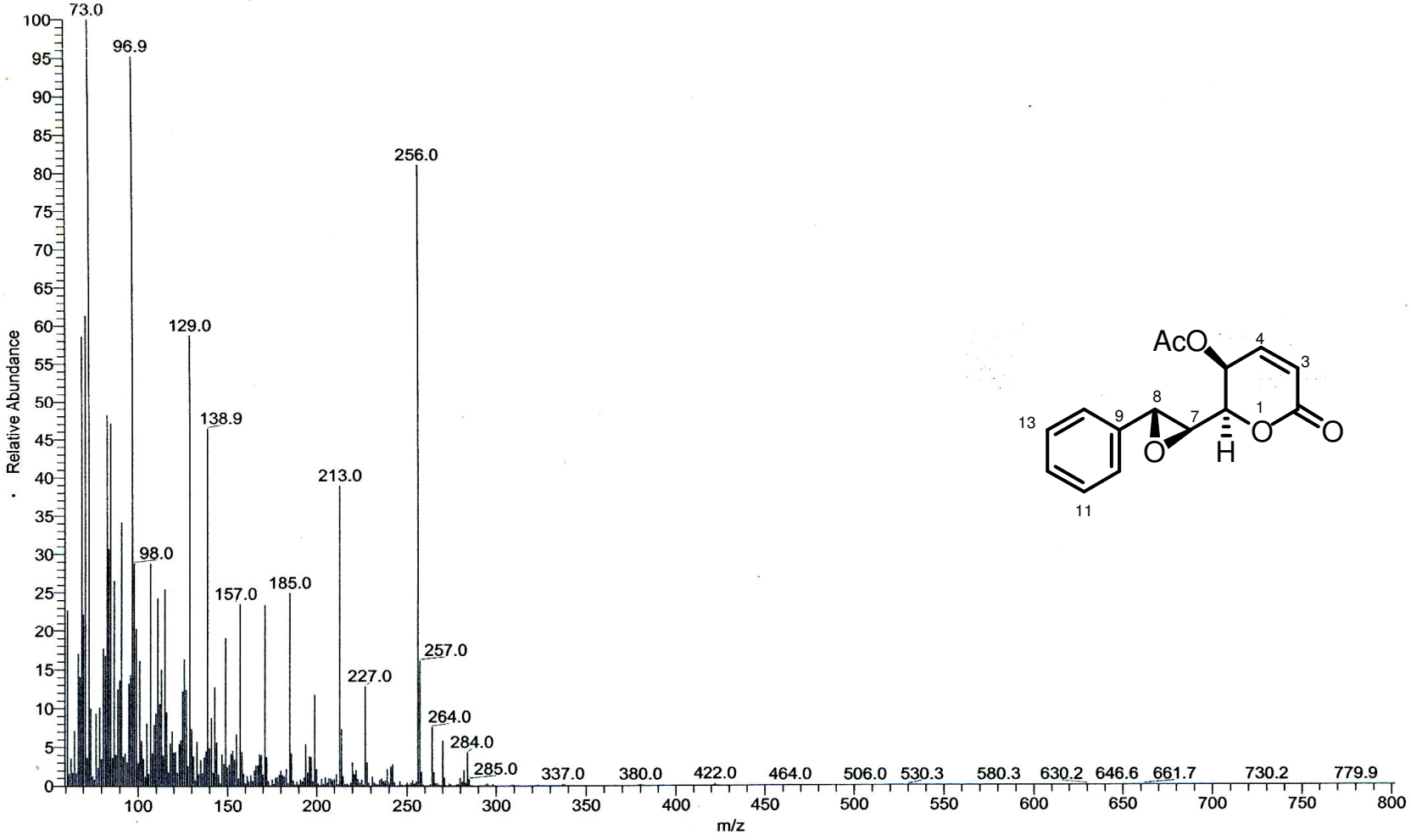
HMQC spectrum of compound 1 (500 MHz, $CDCl_3$)



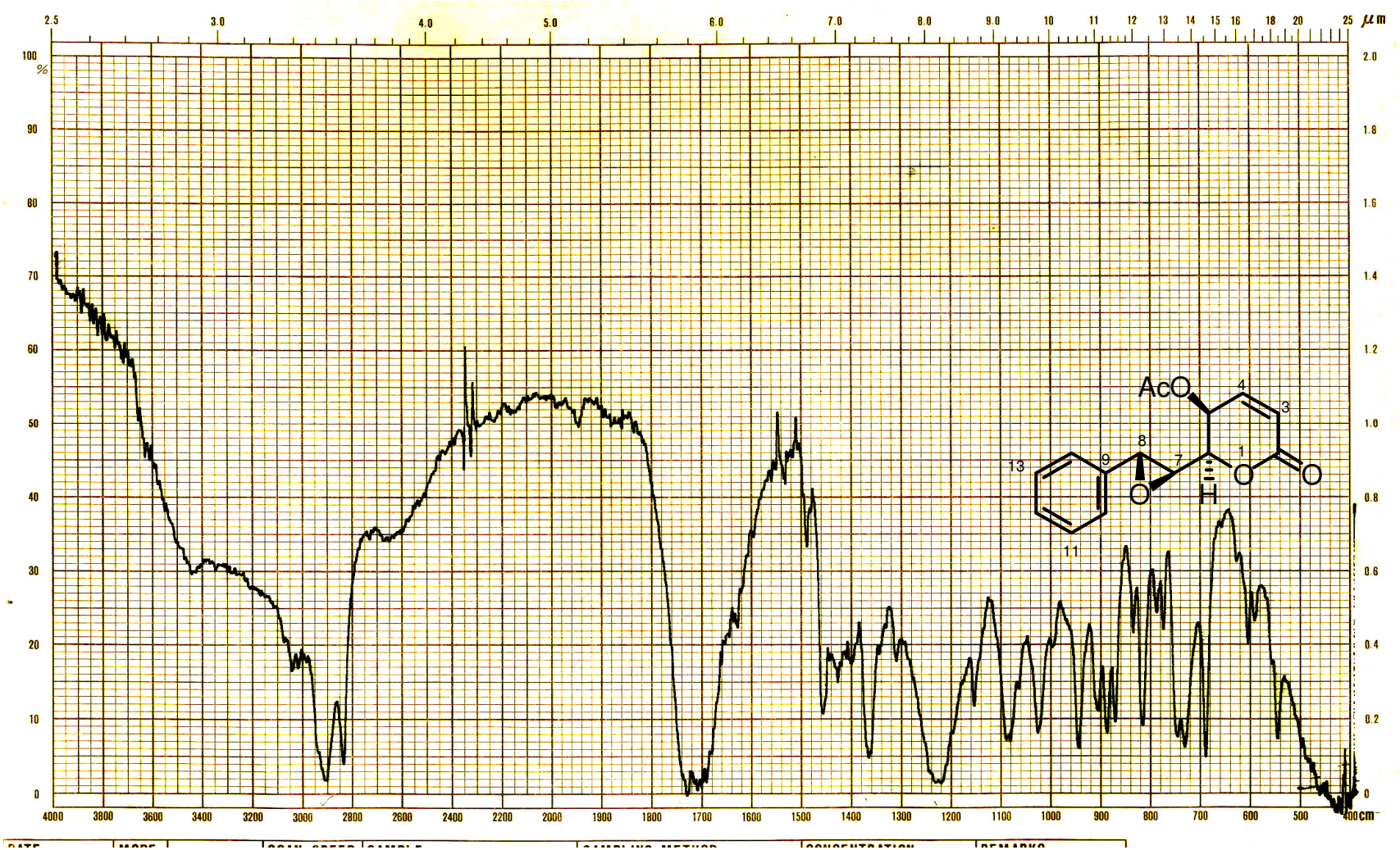
HMBC spectrum of compound 1 (500 MHz, $CDCl_3$)



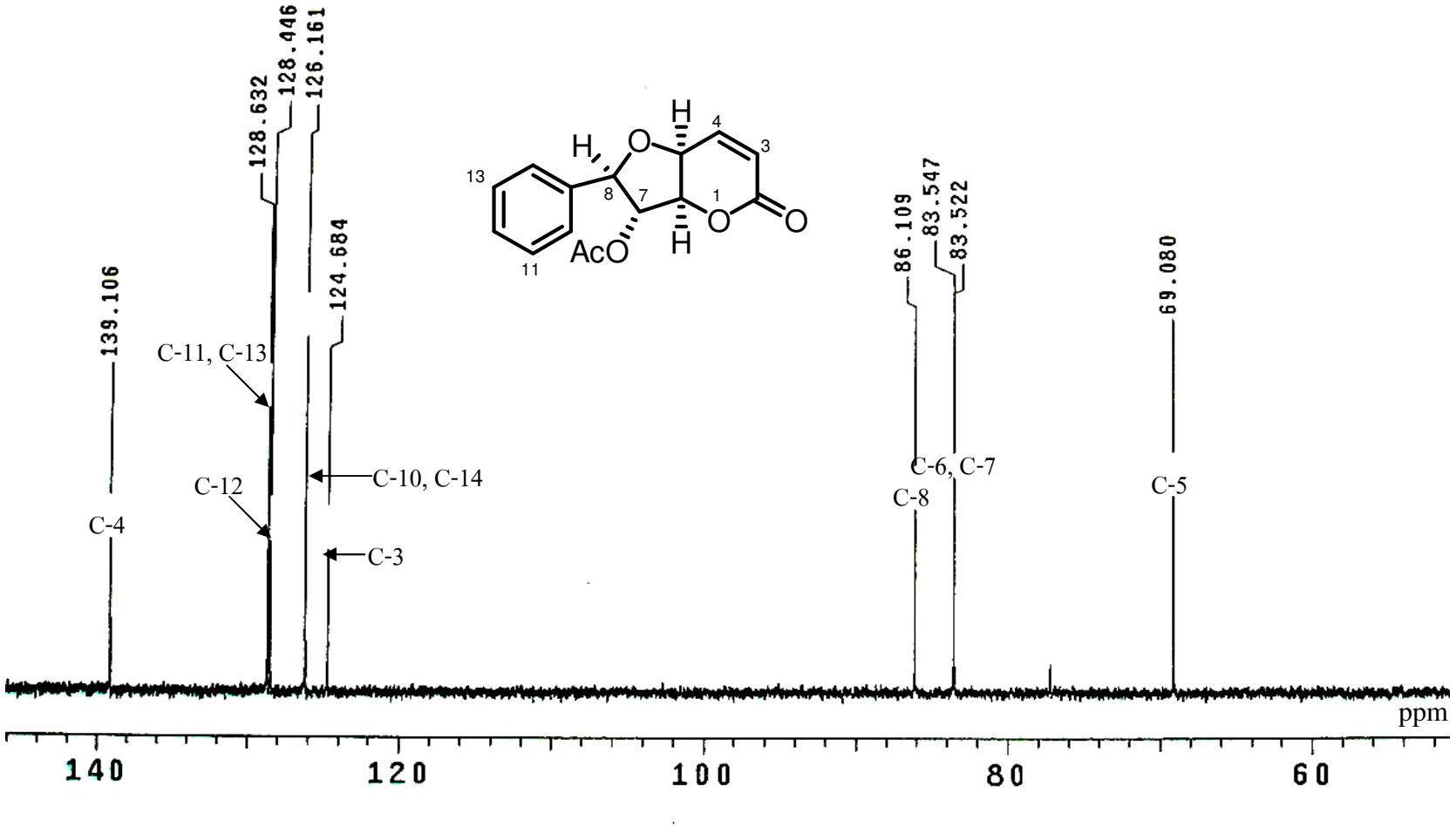
ESIMS spectrum of compound 1



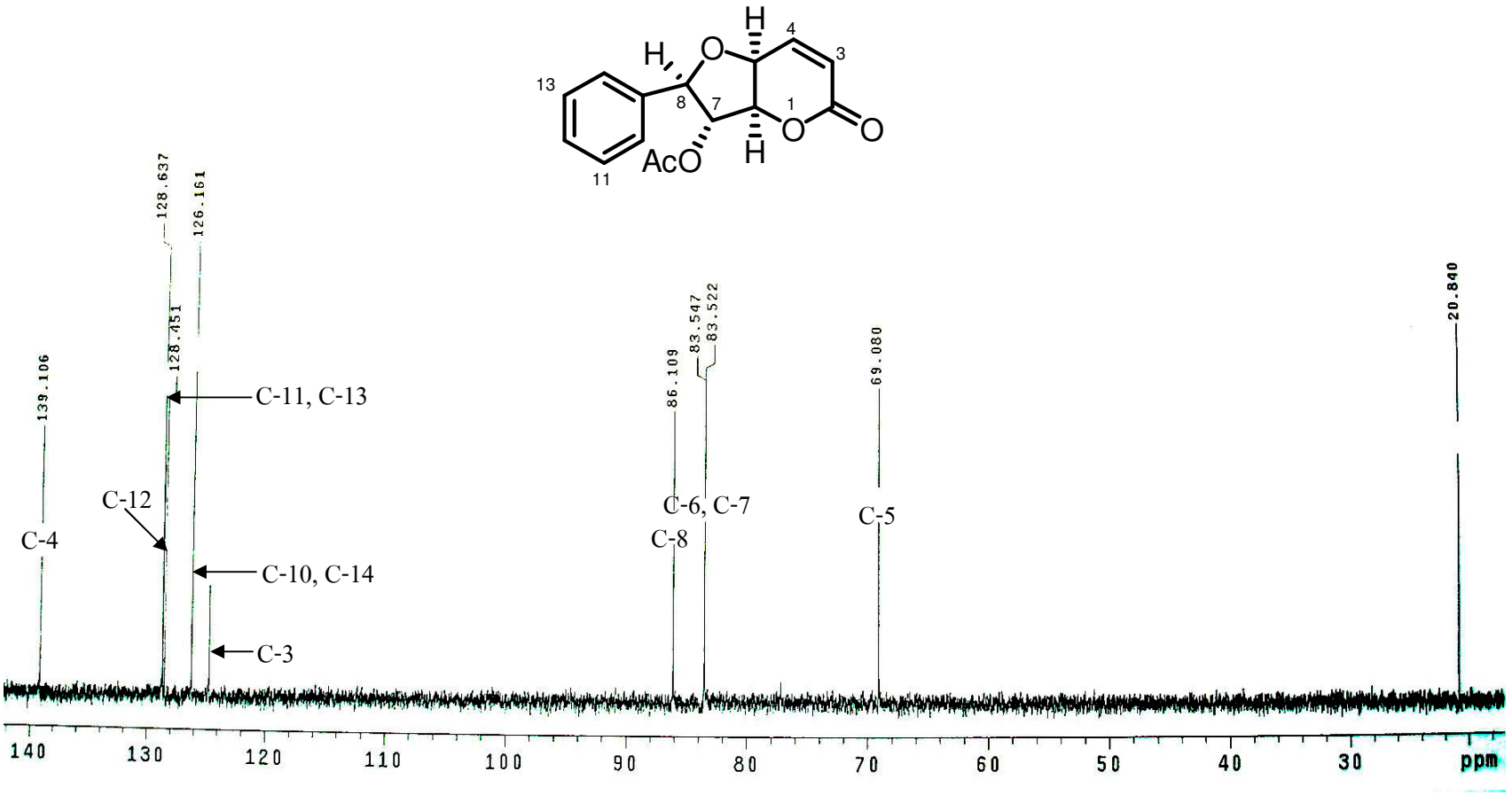
EIMS spectrum of compound 1



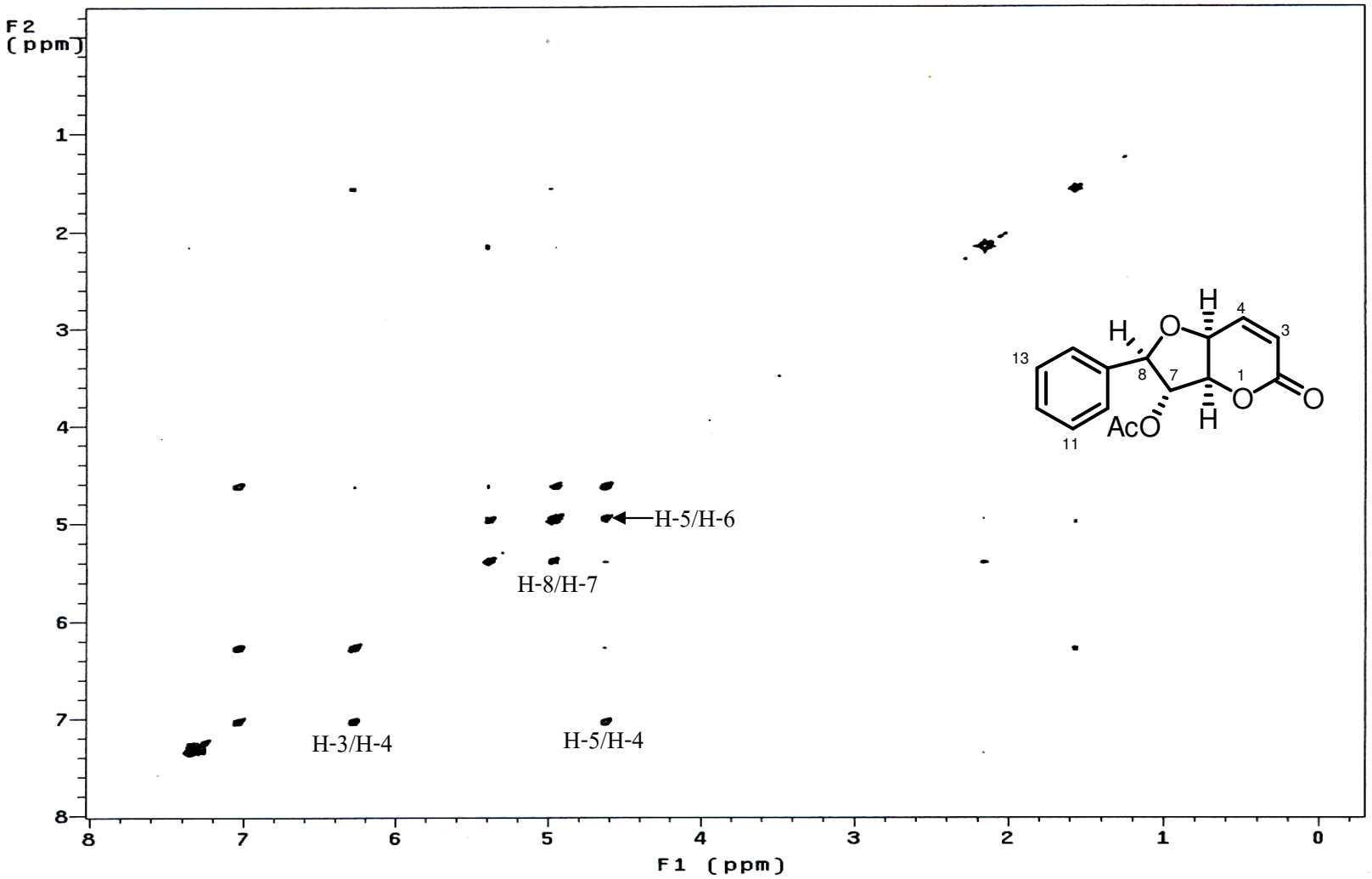
IR spectrum of compound 1



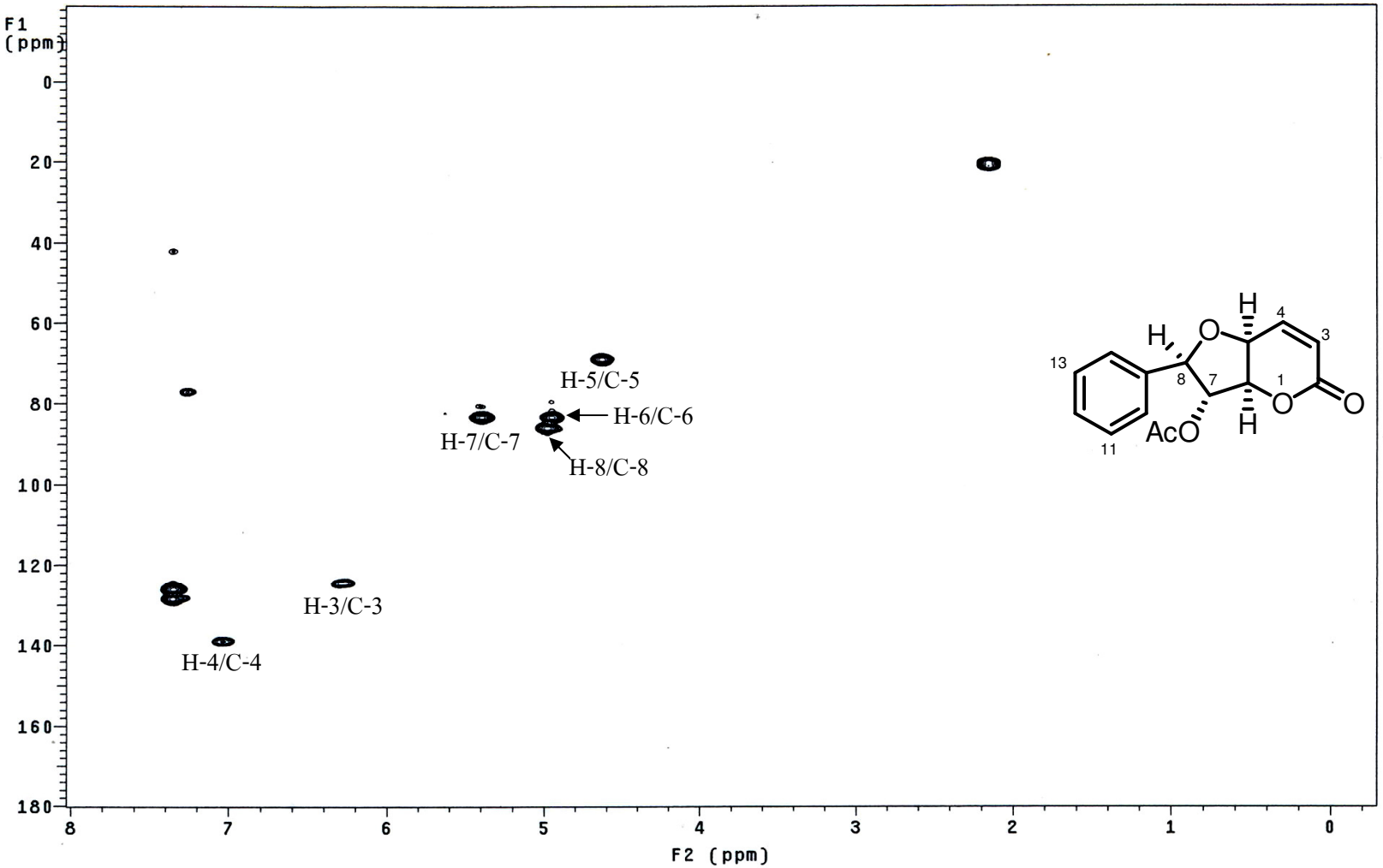
DEPT (90) spectrum of compound 2 in $CDCl_3$



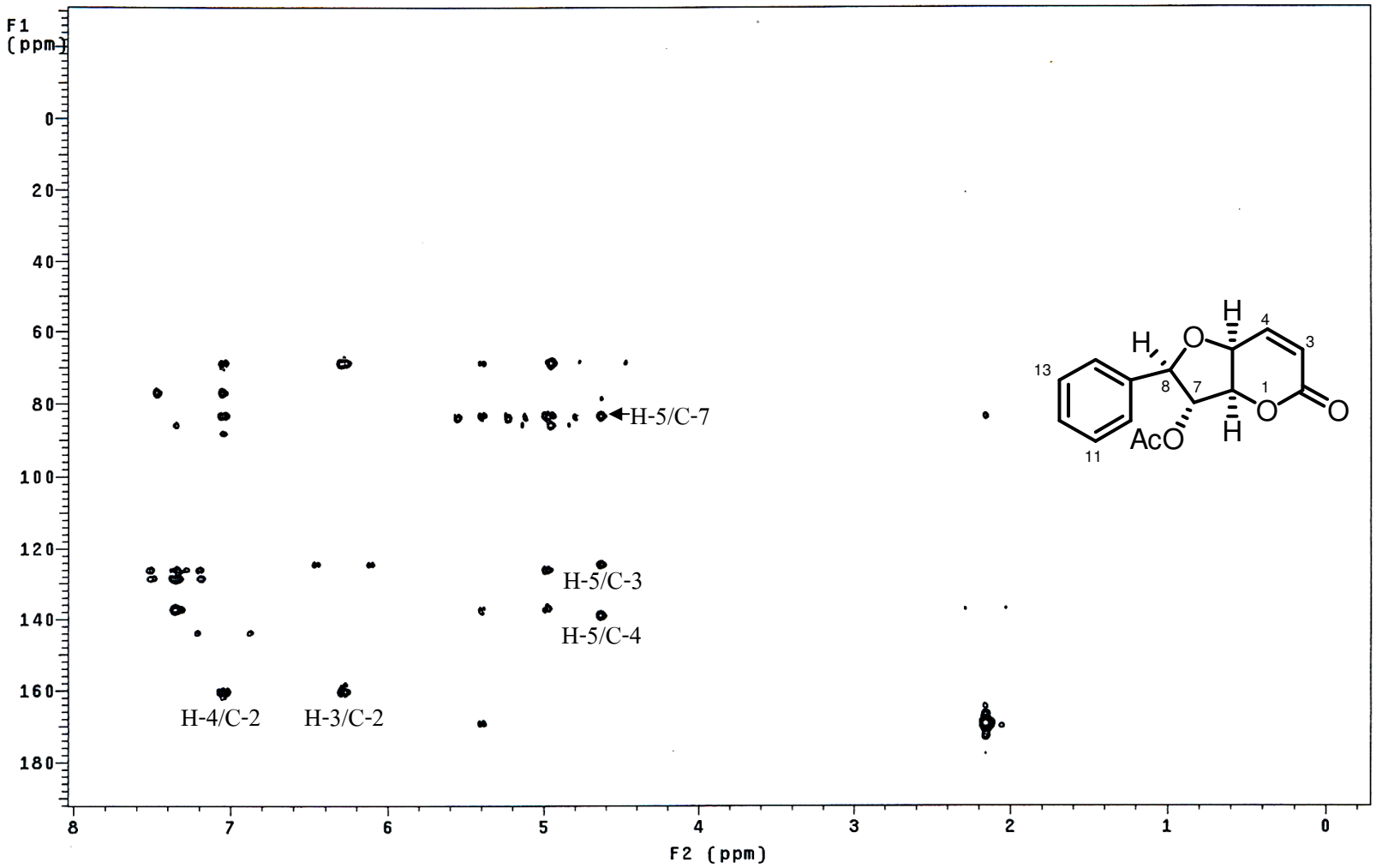
DEPT (135) spectrum of compound 2 in $CDCl_3$



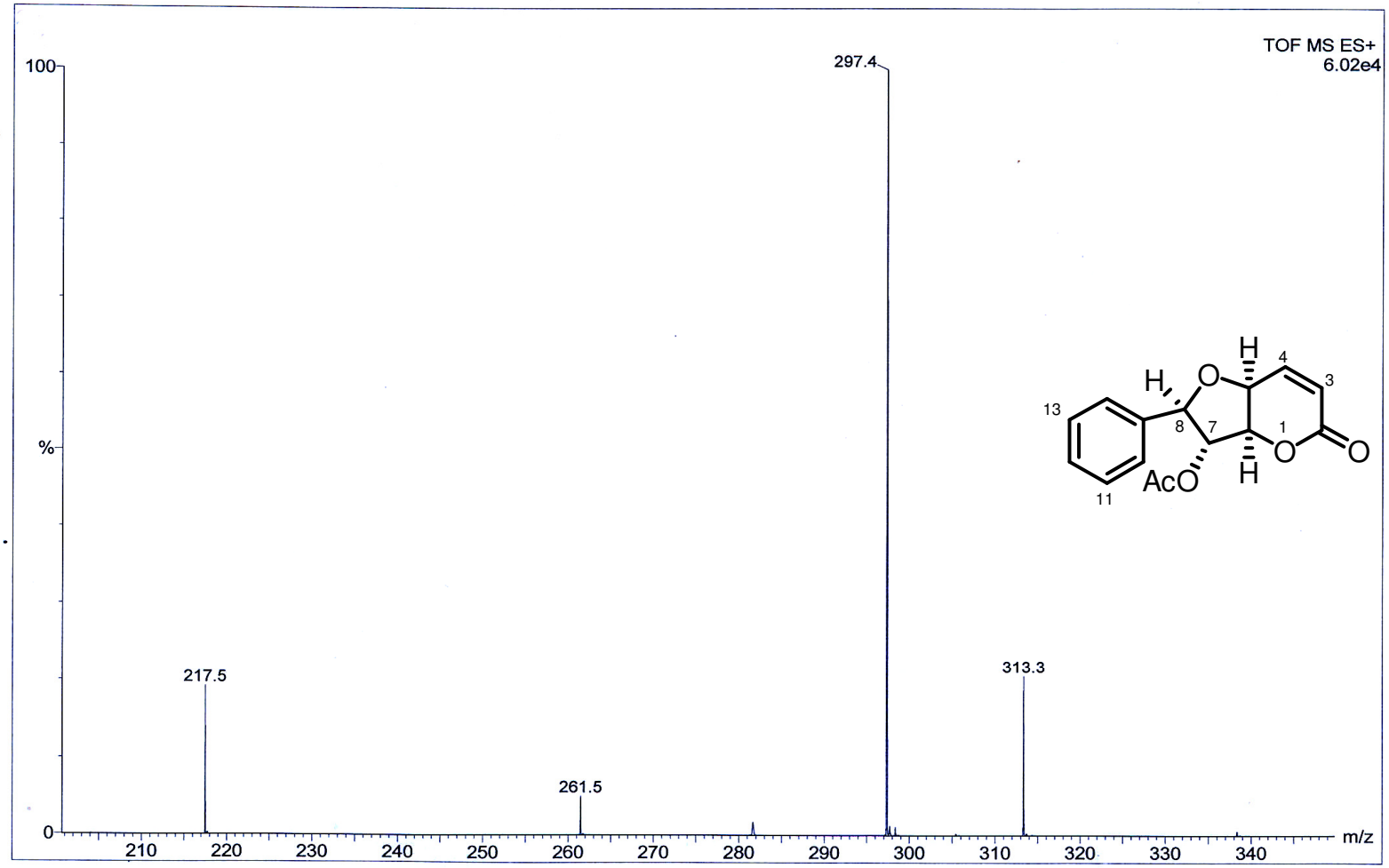
^{1}H-^{1}H COSY spectrum of compound 2 (500 MHz, $CDCl_3$)



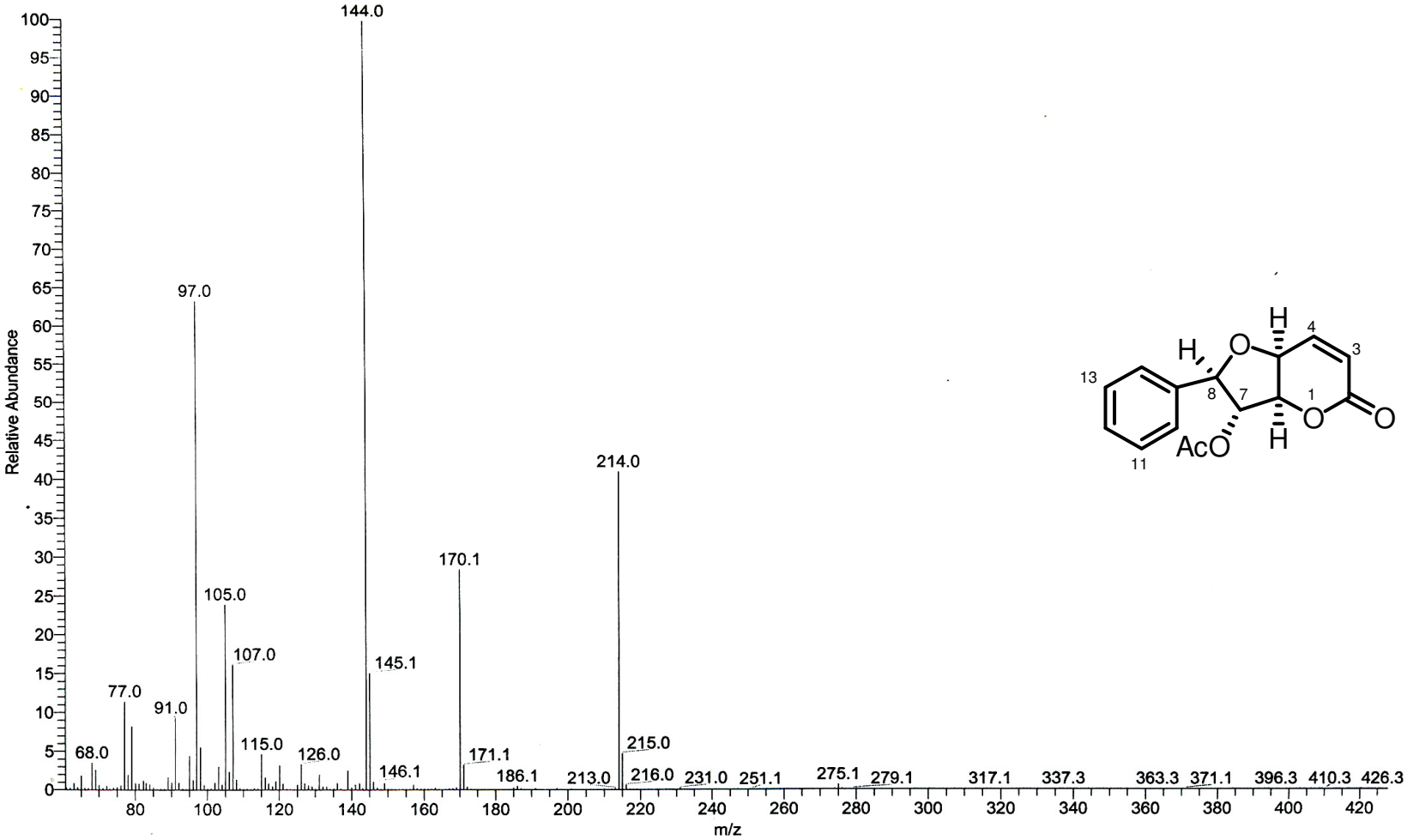
HMQC spectrum of compound 2 (500 MHz, $CDCl_3$)



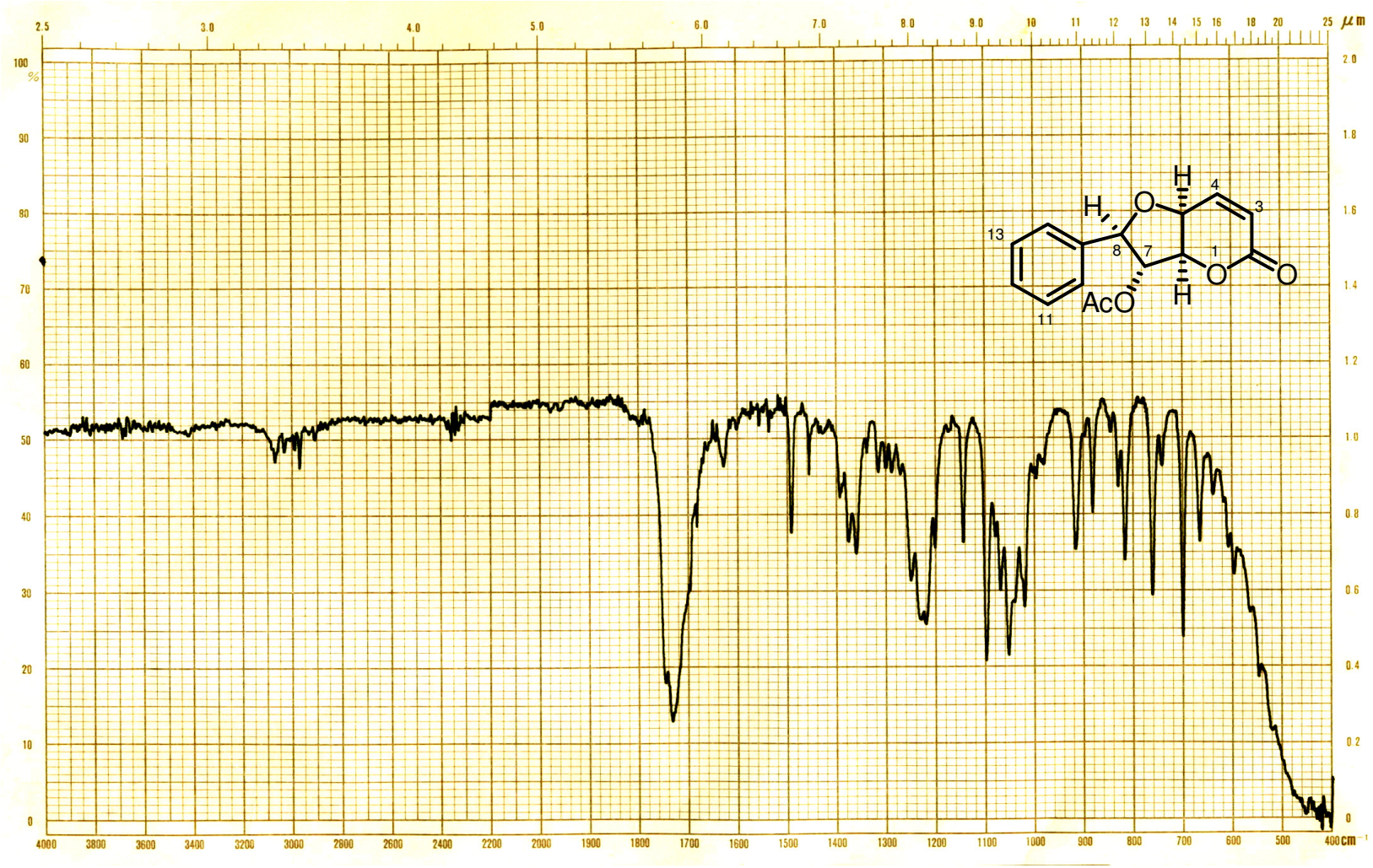
HMBC spectrum of compound 2 (500 MHz, $CDCl_3$)



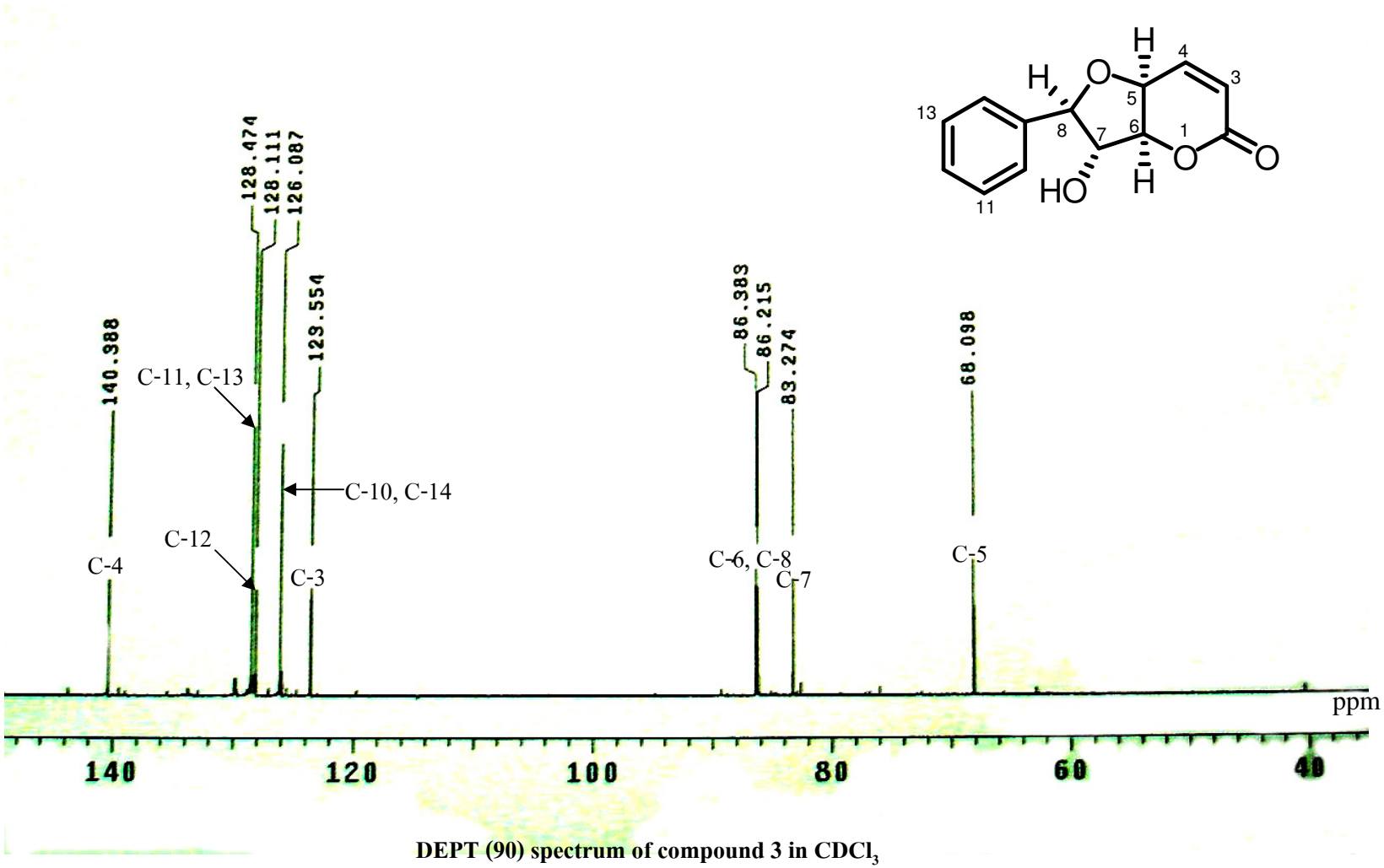
ESIMS spectrum of compound 2



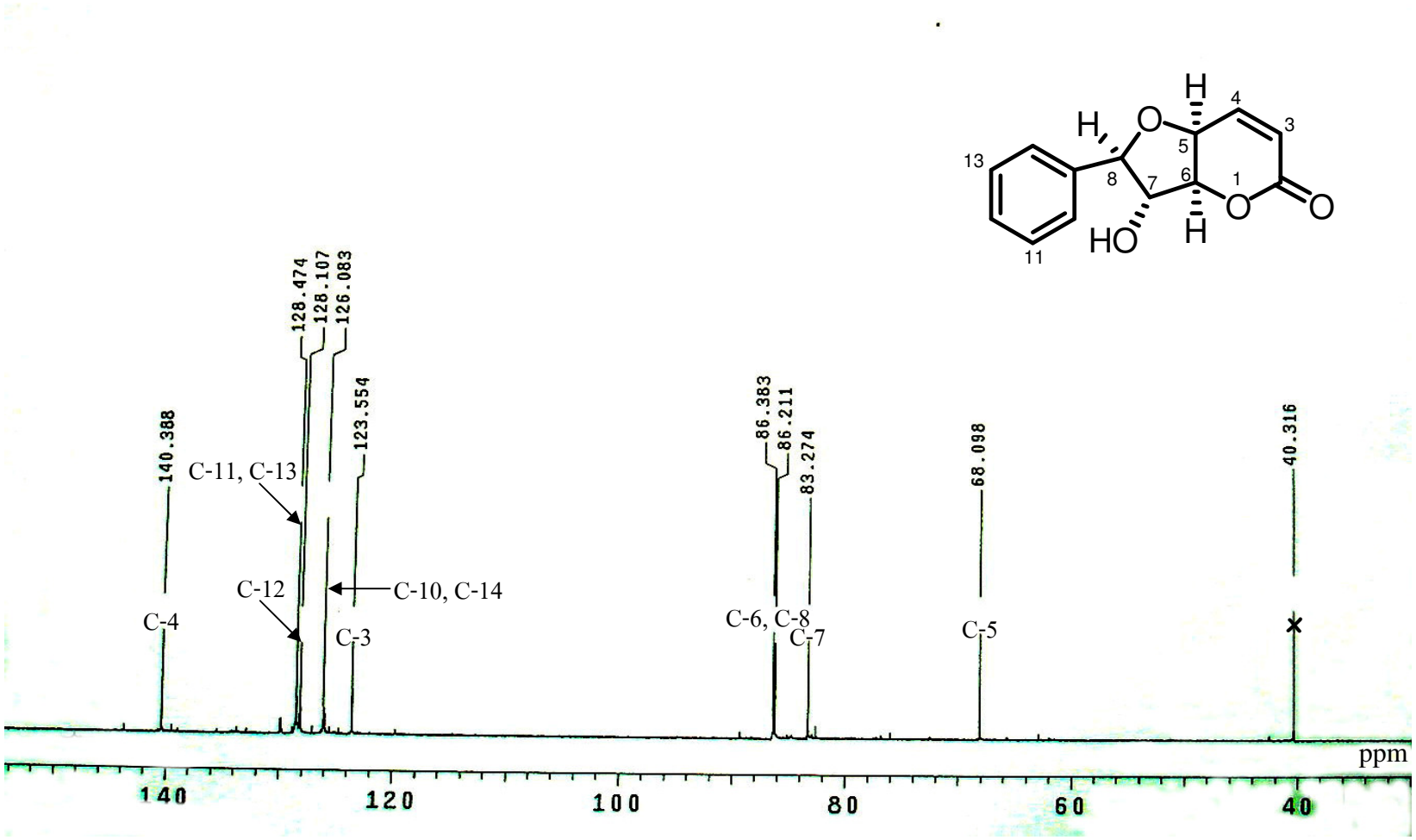
EIMS spectrum of compound 2



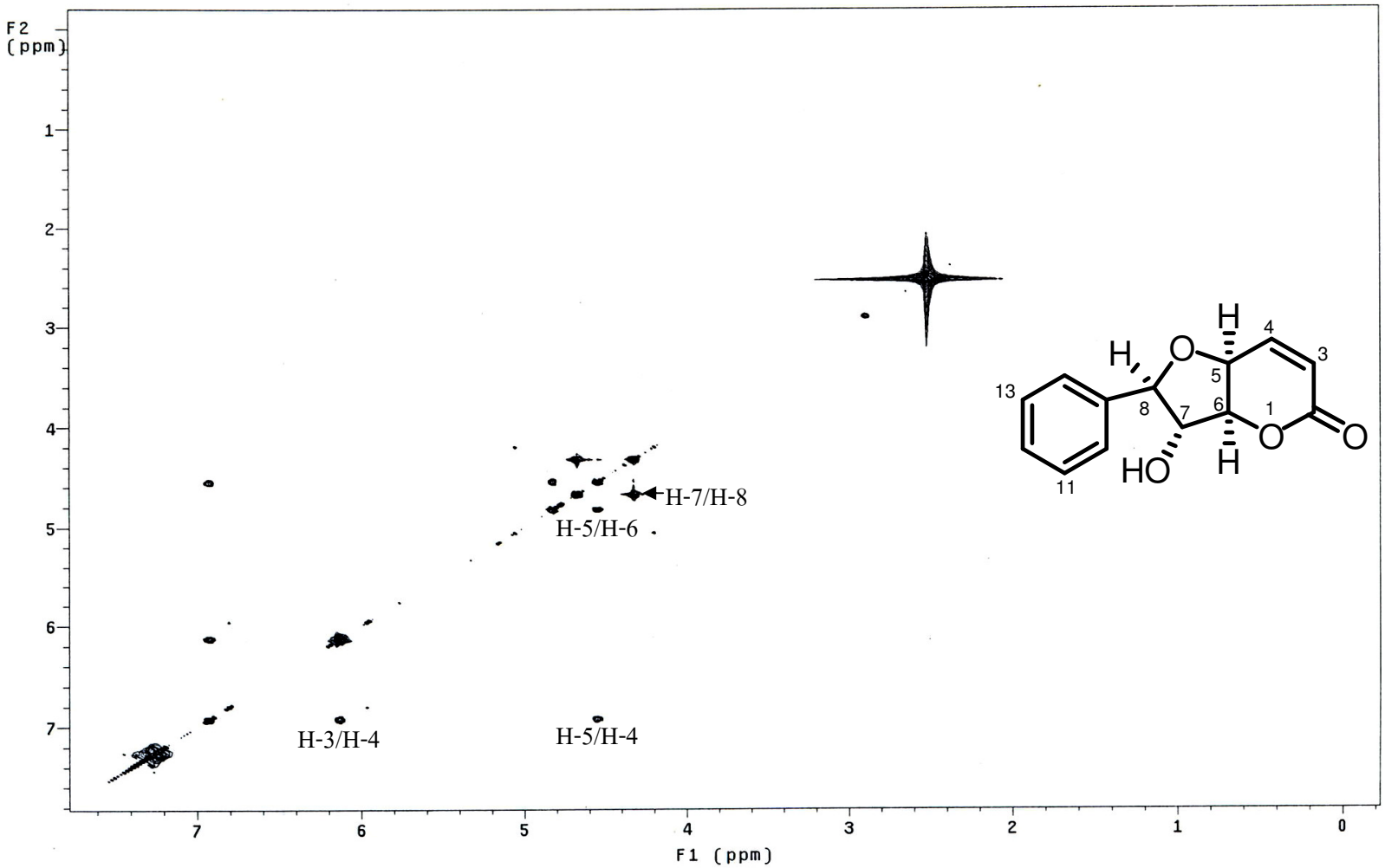
IR spectrum of compound 2



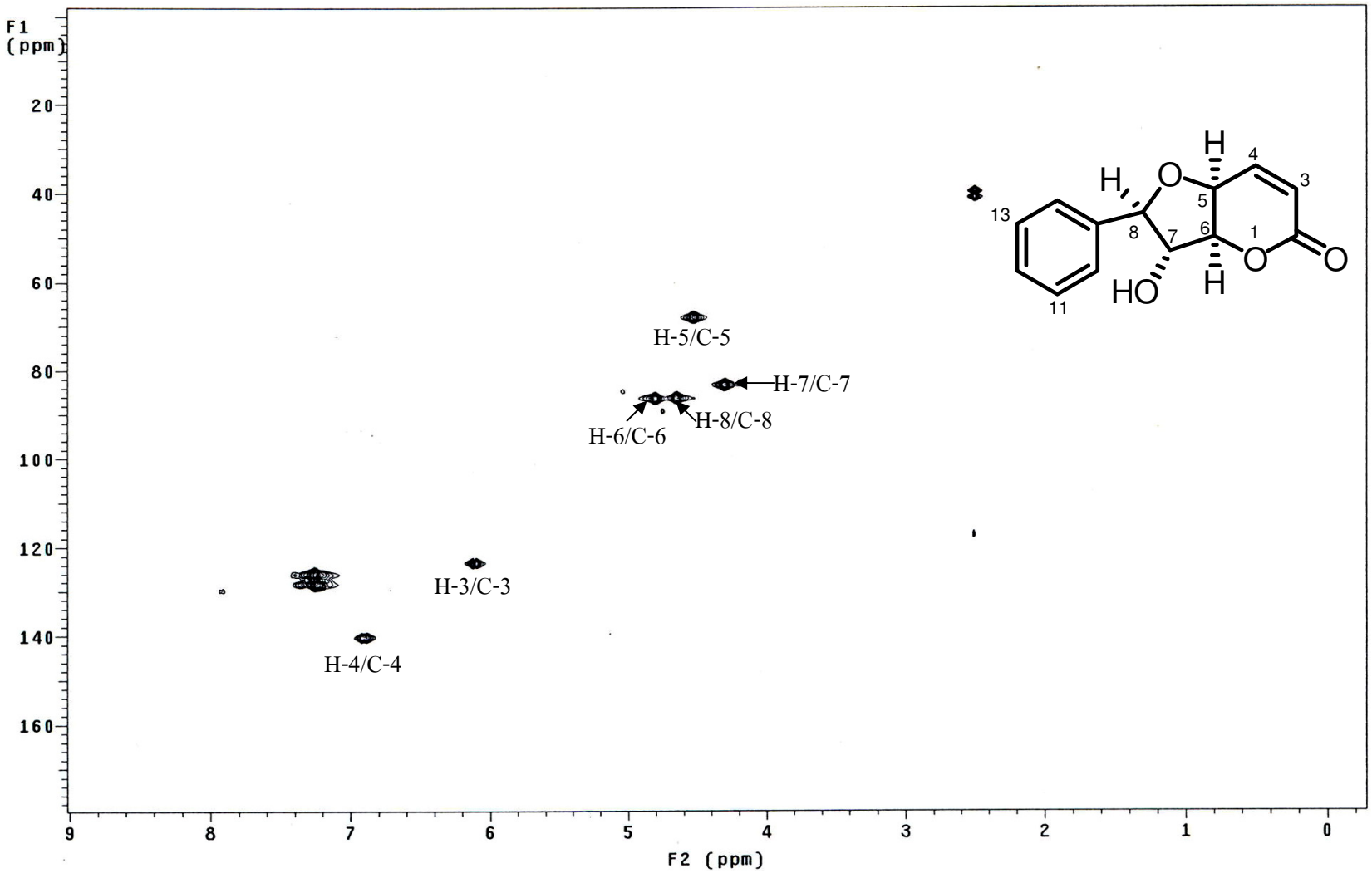
DEPT (90) spectrum of compound 3 in $CDCl_3$



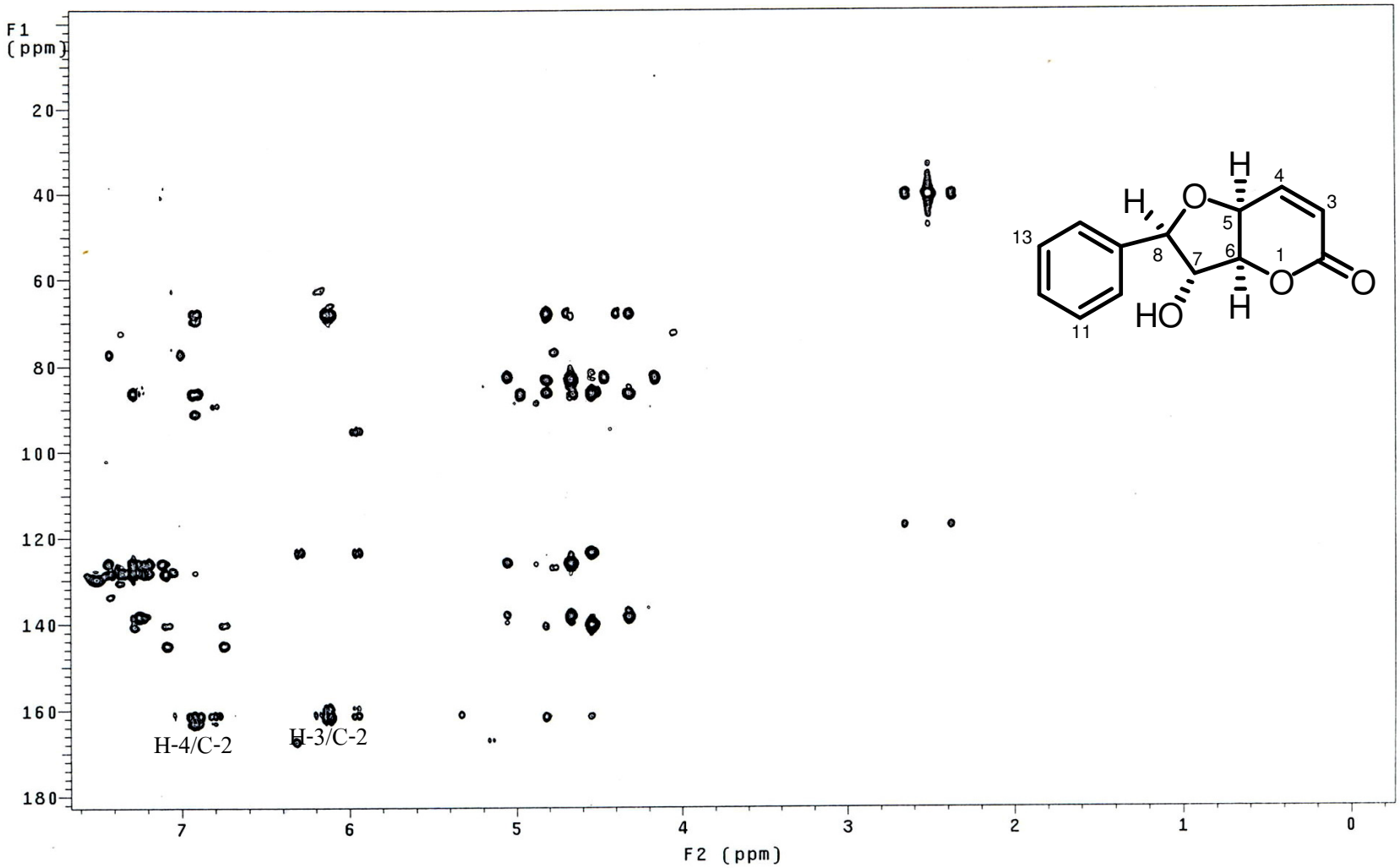
DEPT (135) spectrum of compound 3 in $CDCl_3$



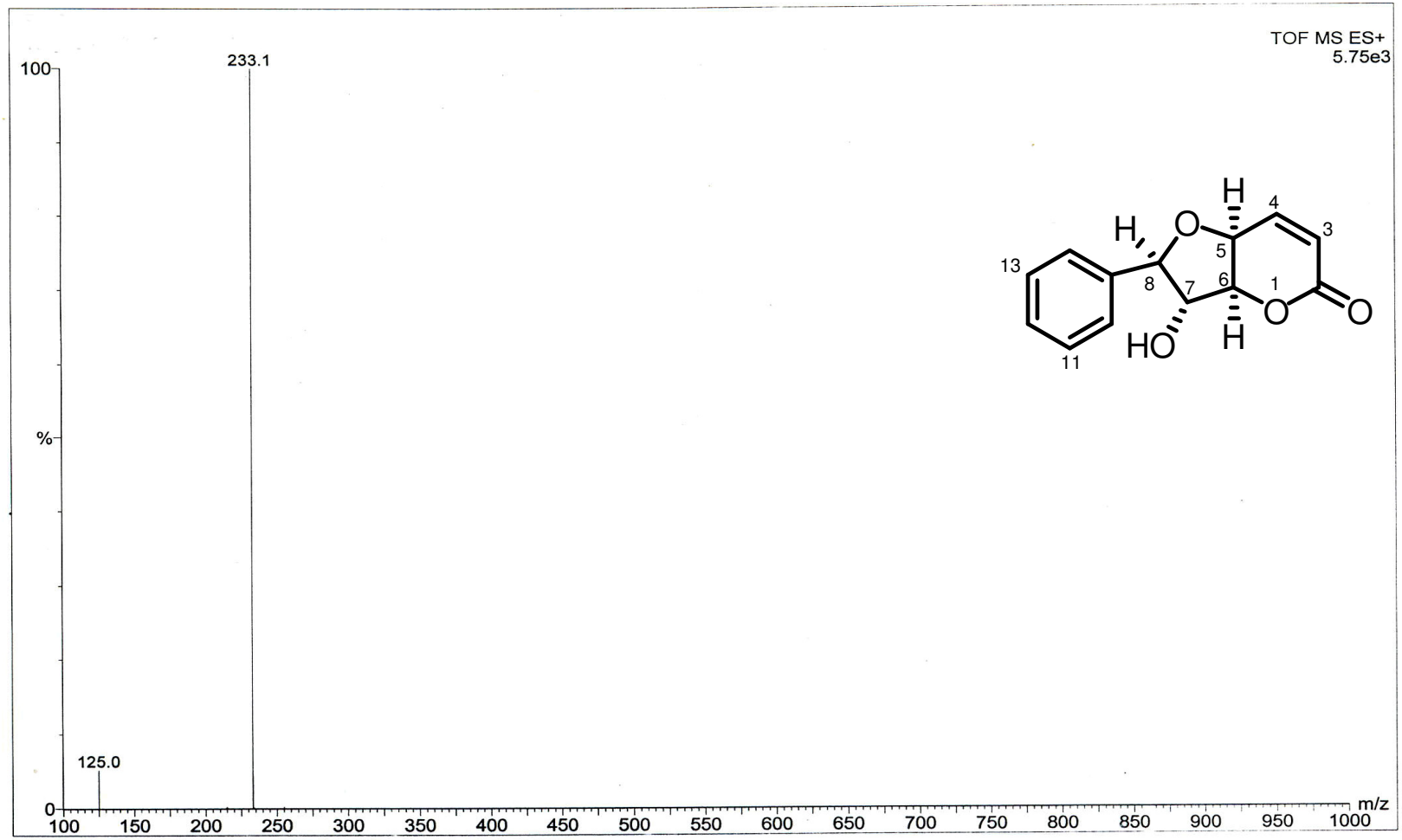
^{1}H-^{1}H COSY spectrum of compound 3 (500 MHz, $CDCl_3$)



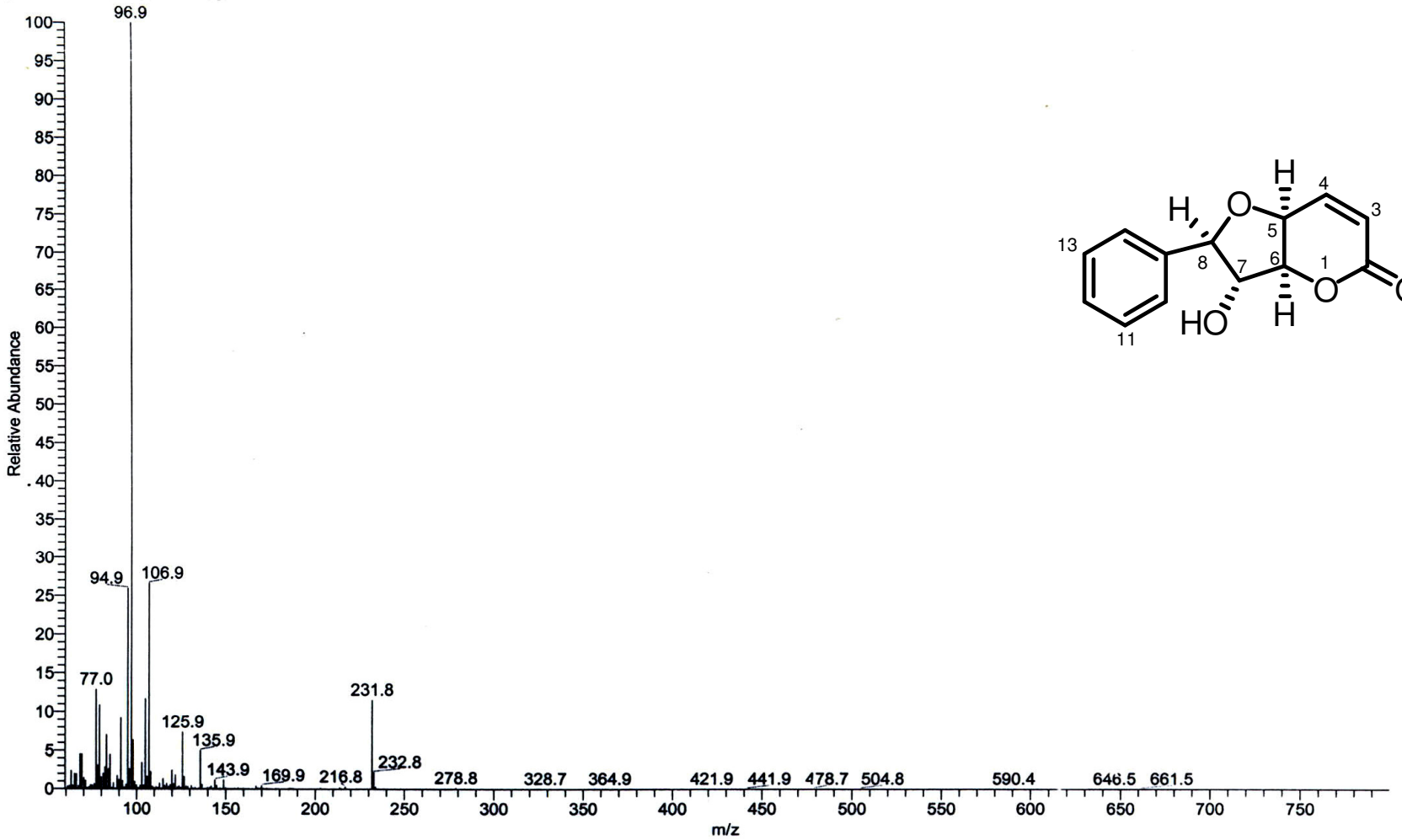
HMQC spectrum of compound 3 (500 MHz, $CDCl_3$)



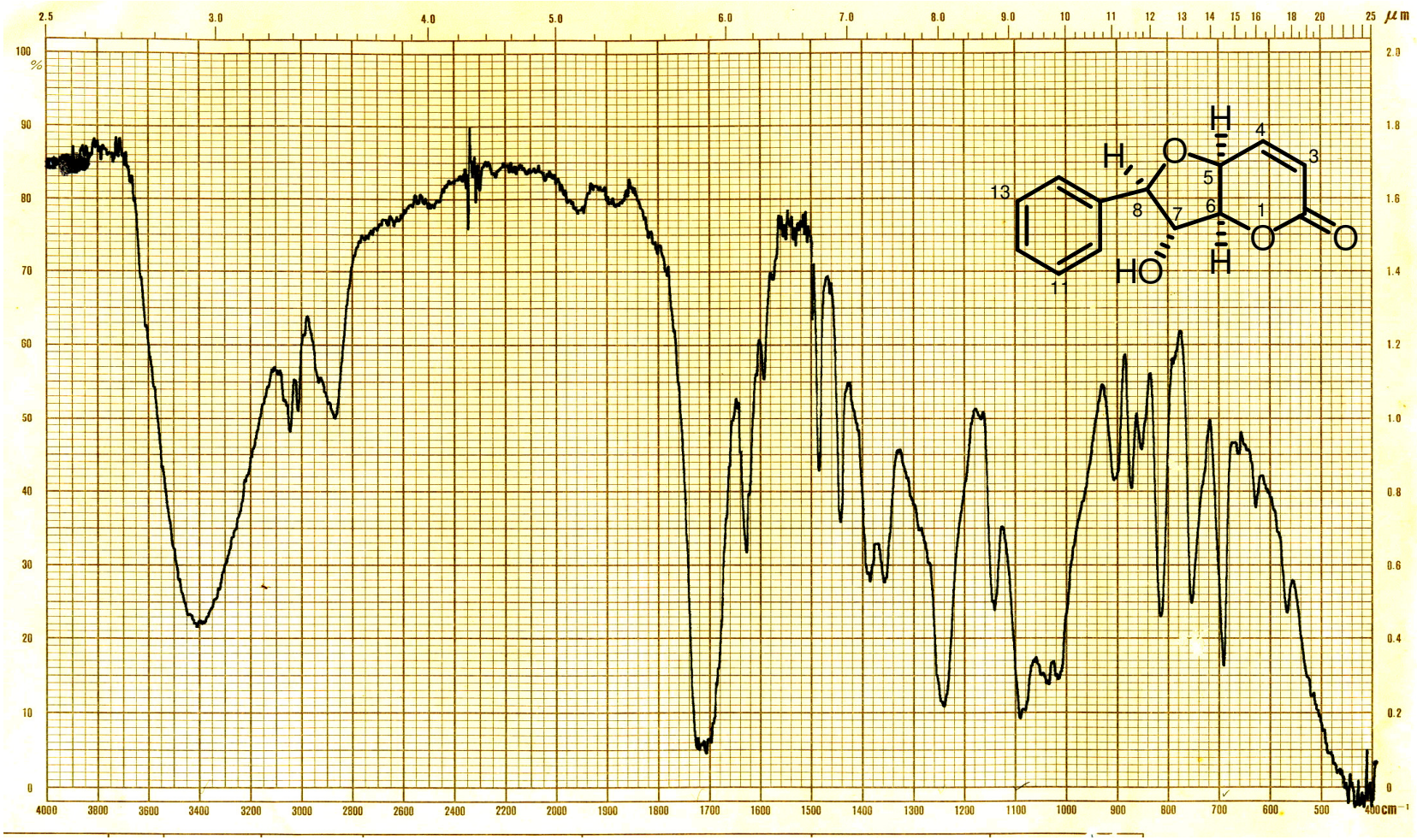
HMBC spectrum of compound 3 (500 MHz, $CDCl_3$)



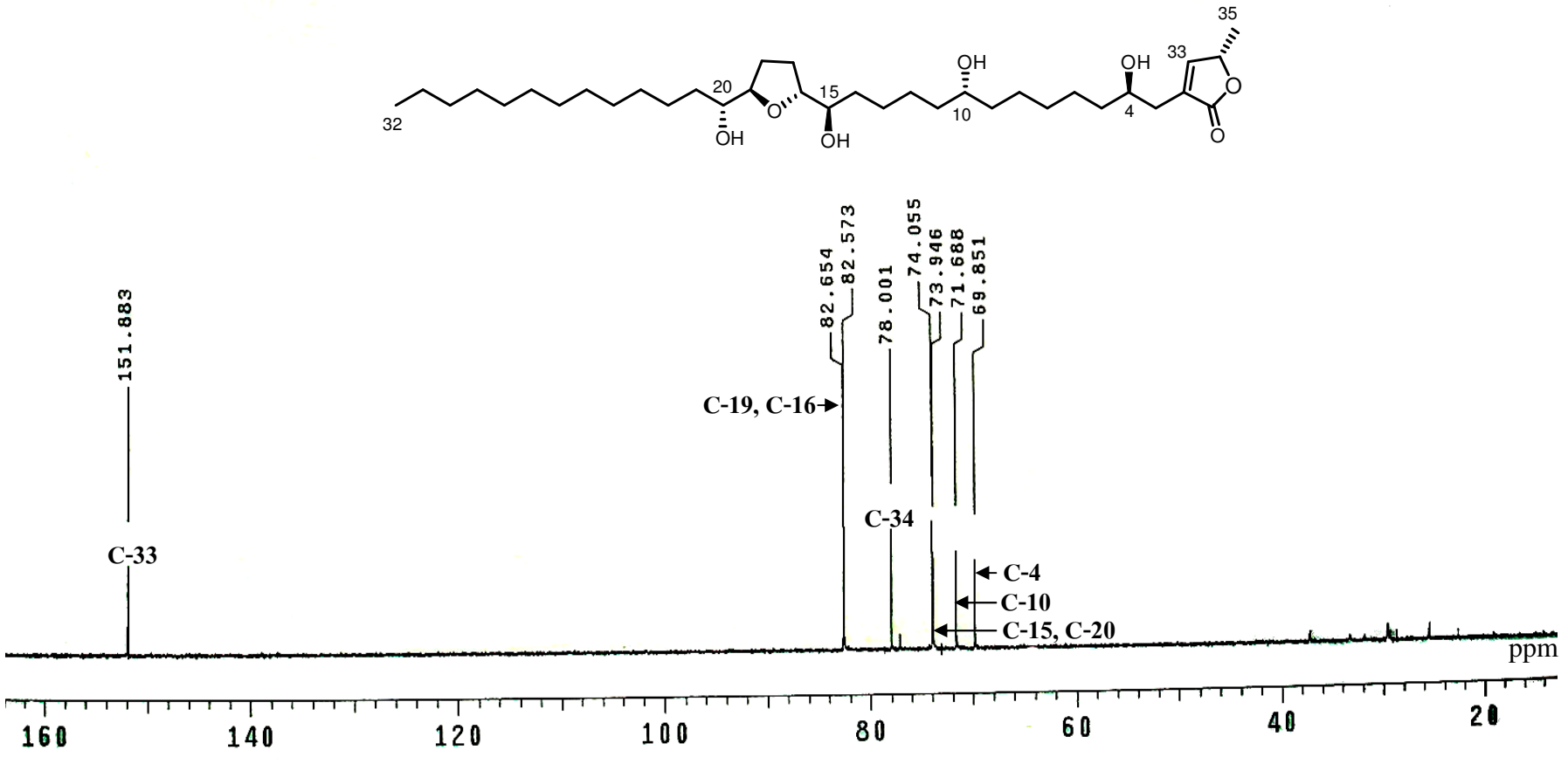
ESIMS spectrum of compound 3



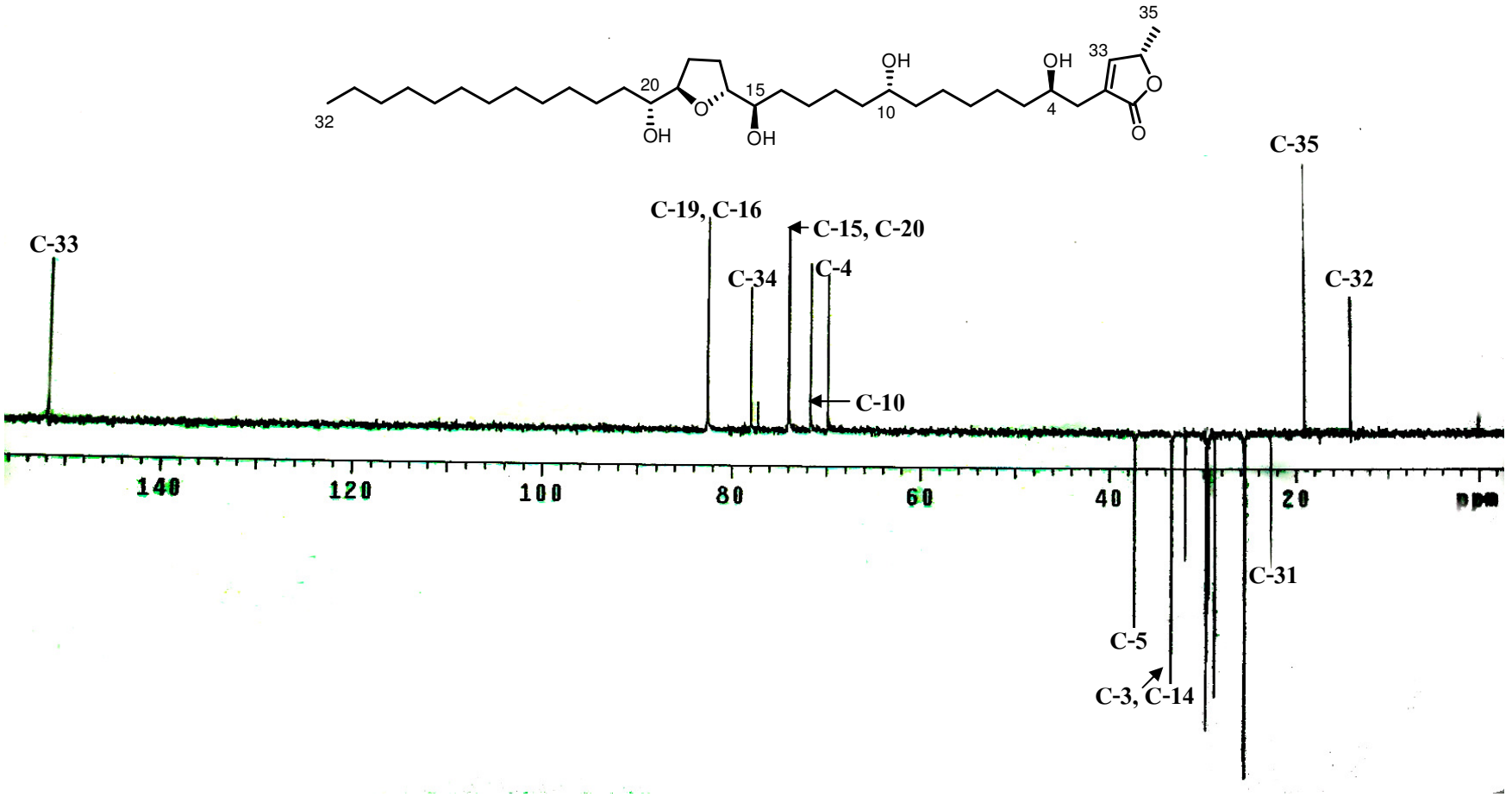
EIMS spectrum of compound 3



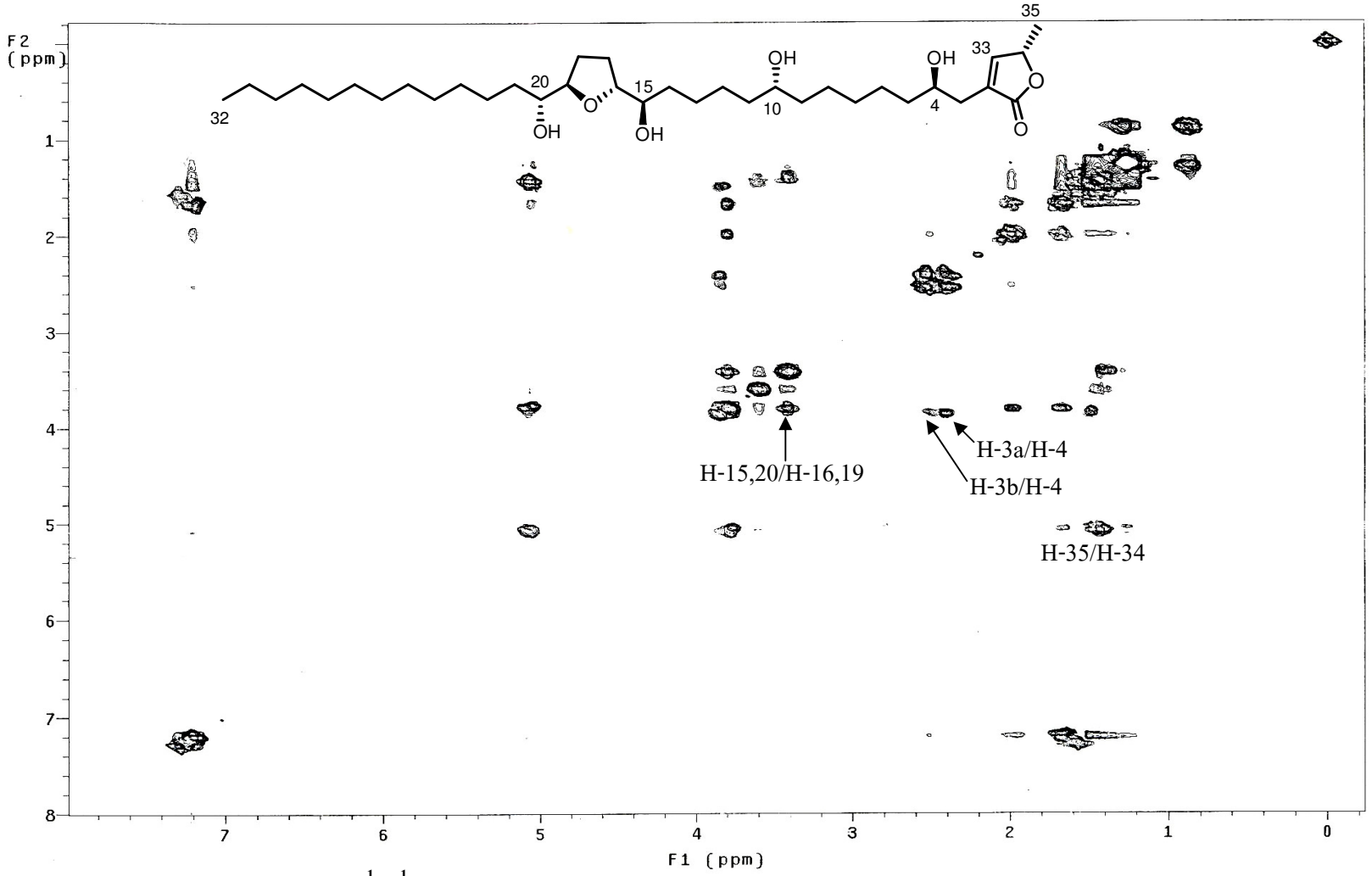
IR spectrum of compound 3



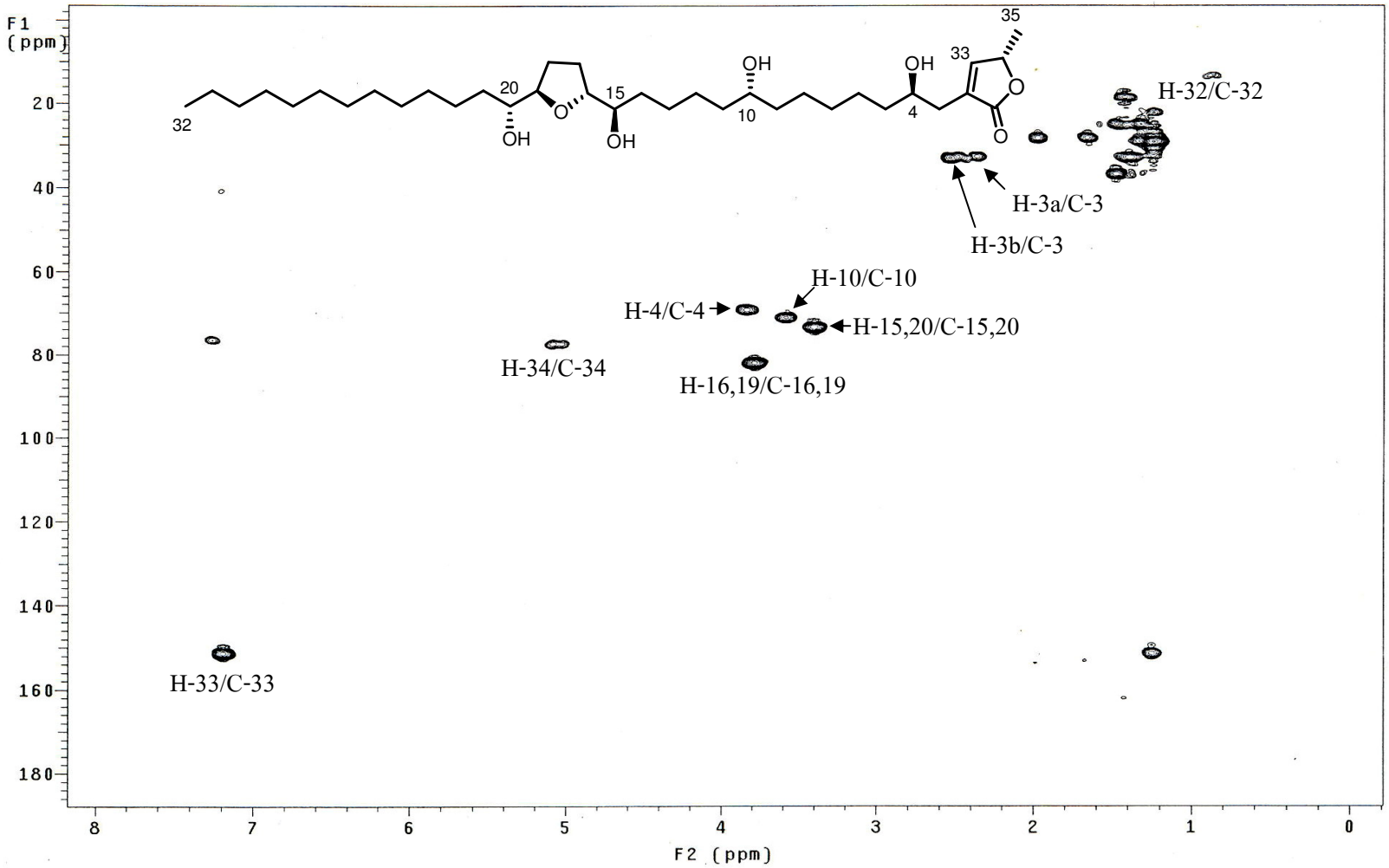
DEPT (90) spectrum of compound 4 in $CDCl_3$



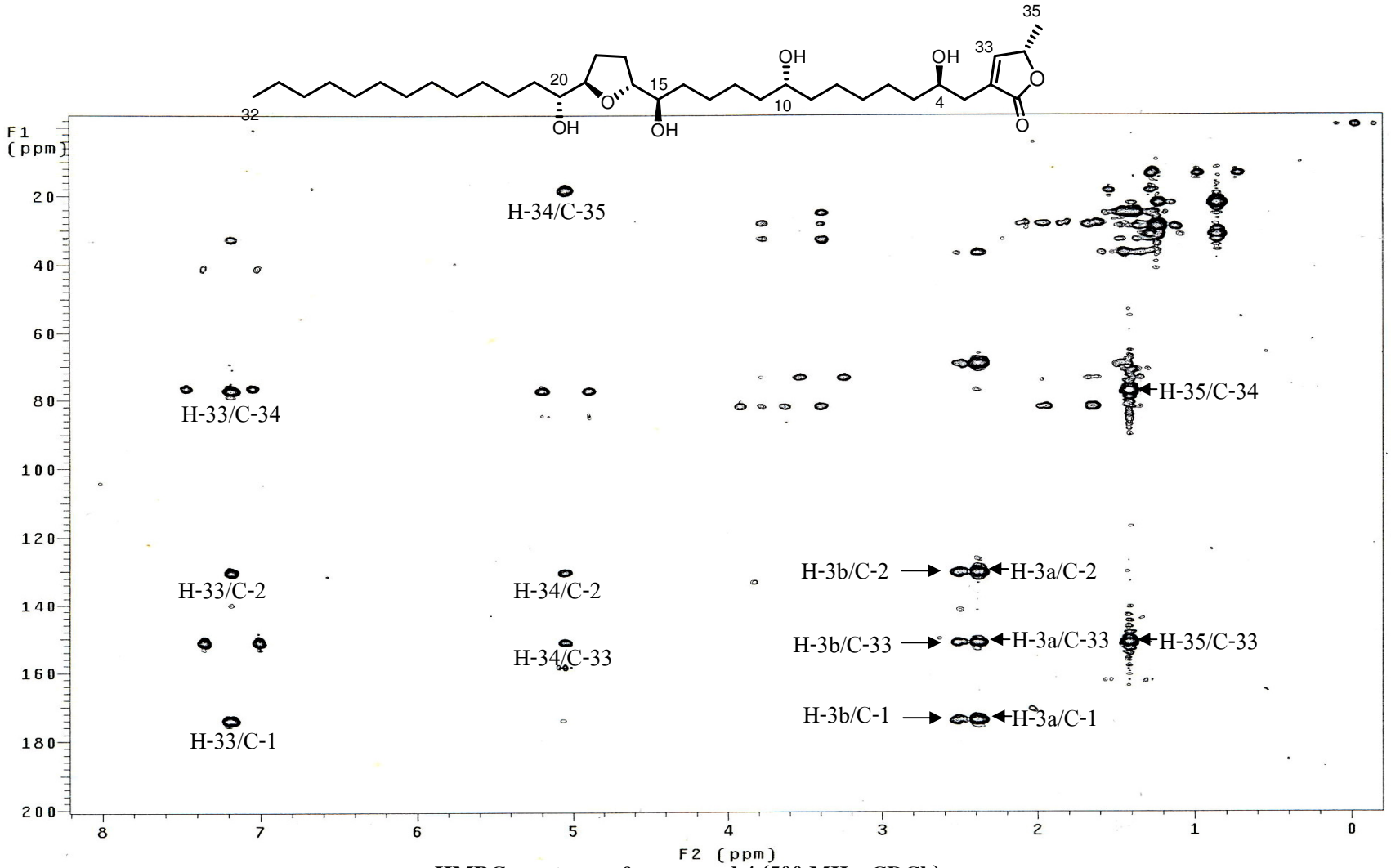
DEPT (135) spectrum of compound 4 in $CDCl_3$



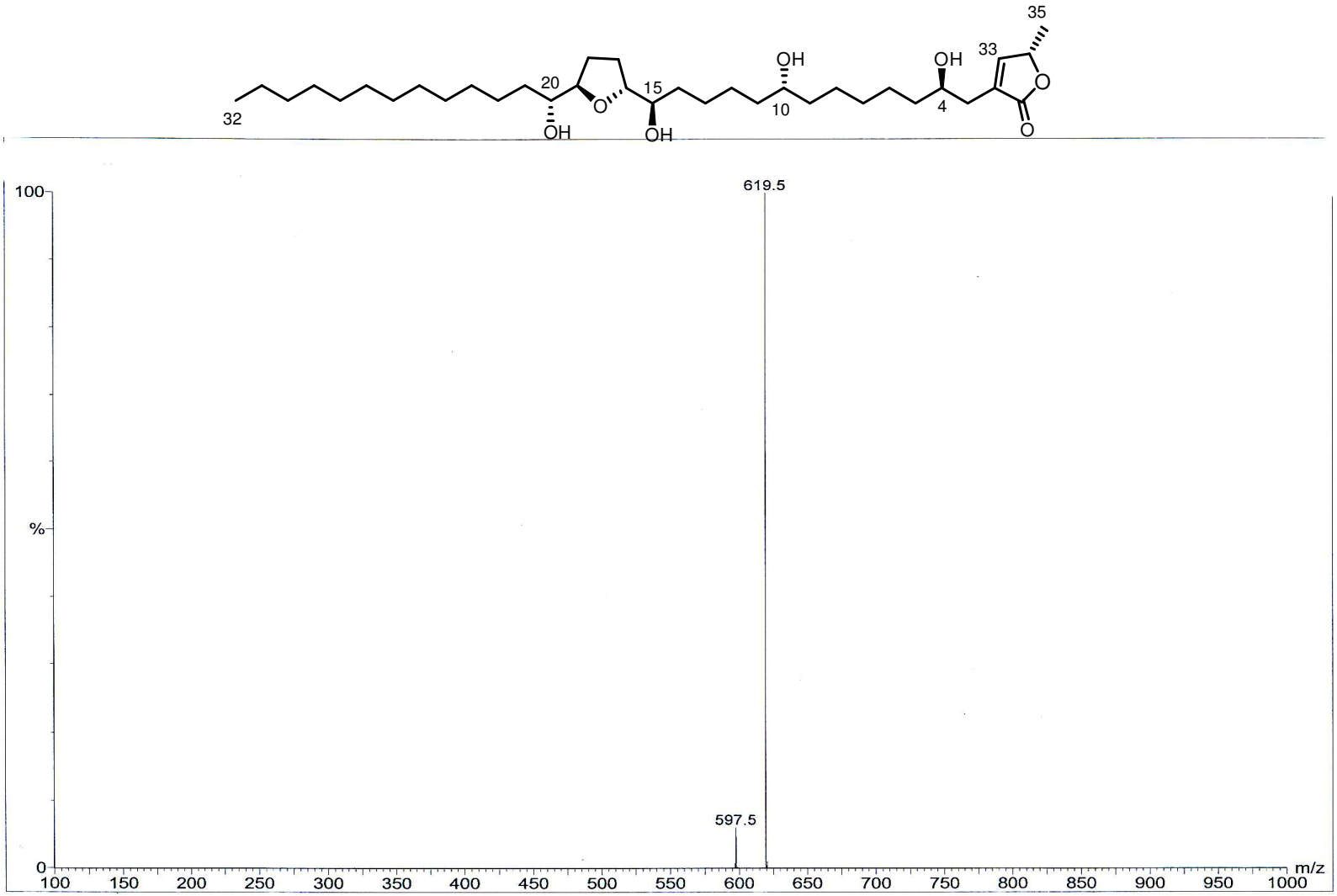
^{1}H-^{1}H COSY spectrum of compound 4 (500 MHz, $CDCl_3$)



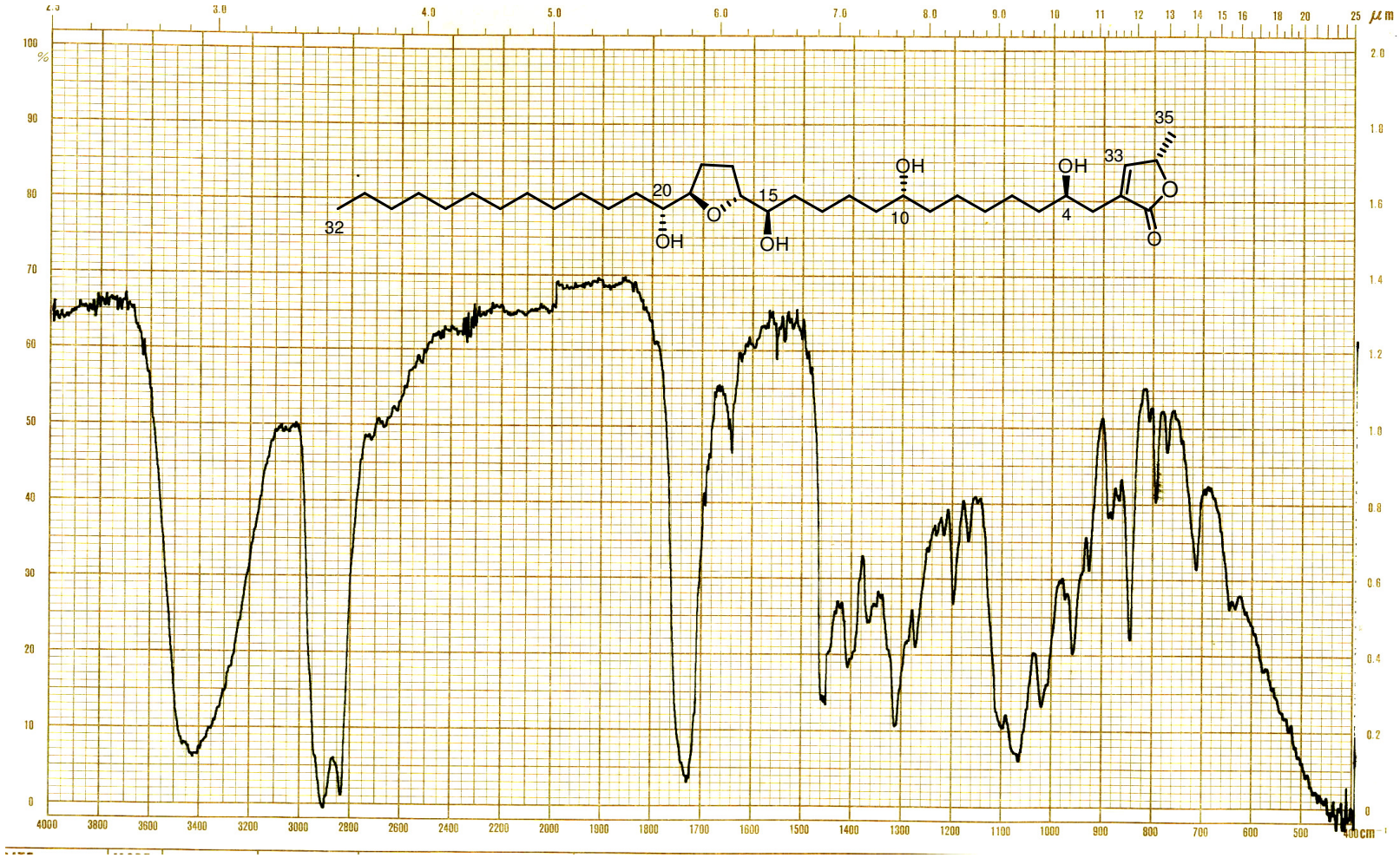
HMQC spectrum of compound 4 (500 MHz, $CDCl_3$)



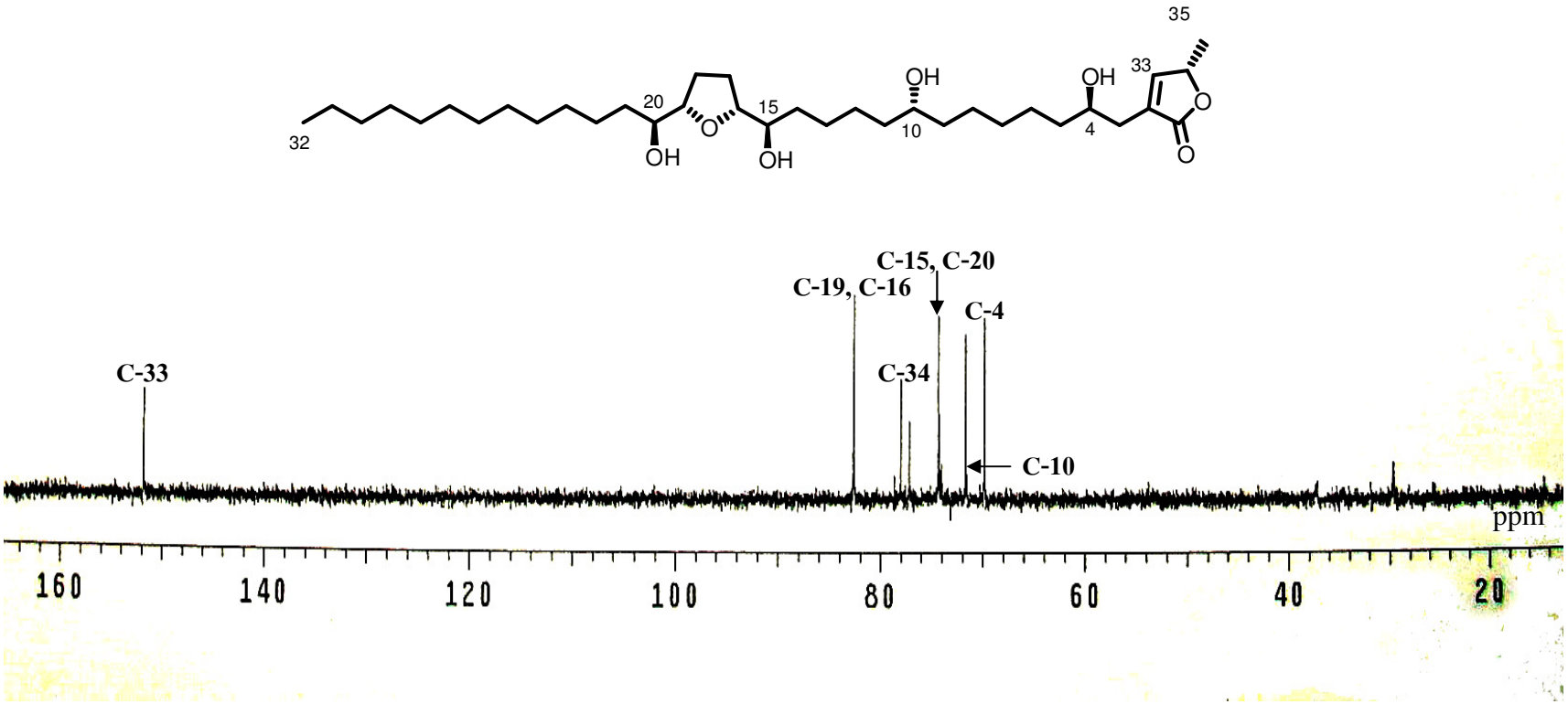
HMBC spectrum of compound 4 (500 MHz, $CDCl_3$)



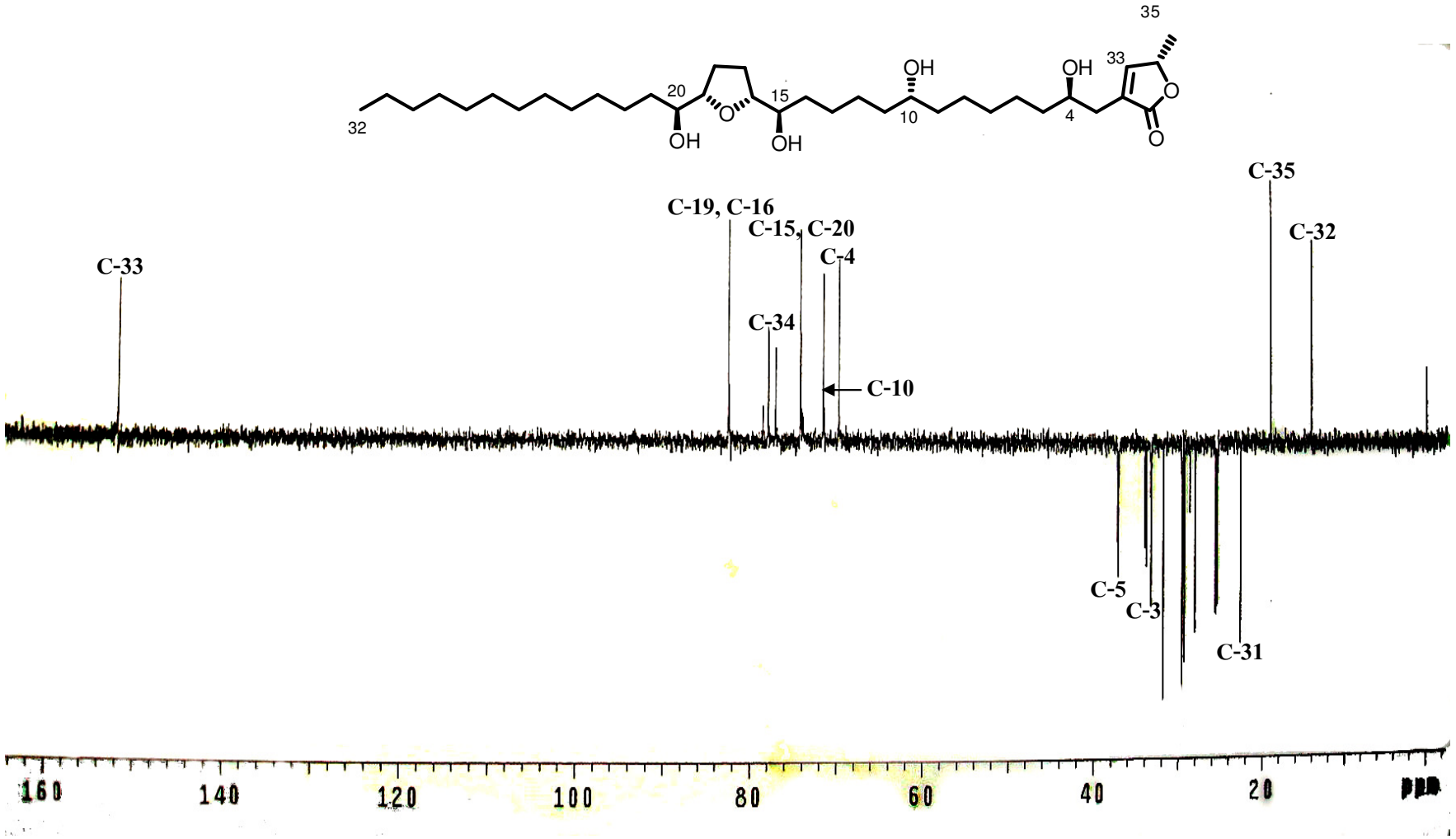
ESIMS spectrum of compound 4



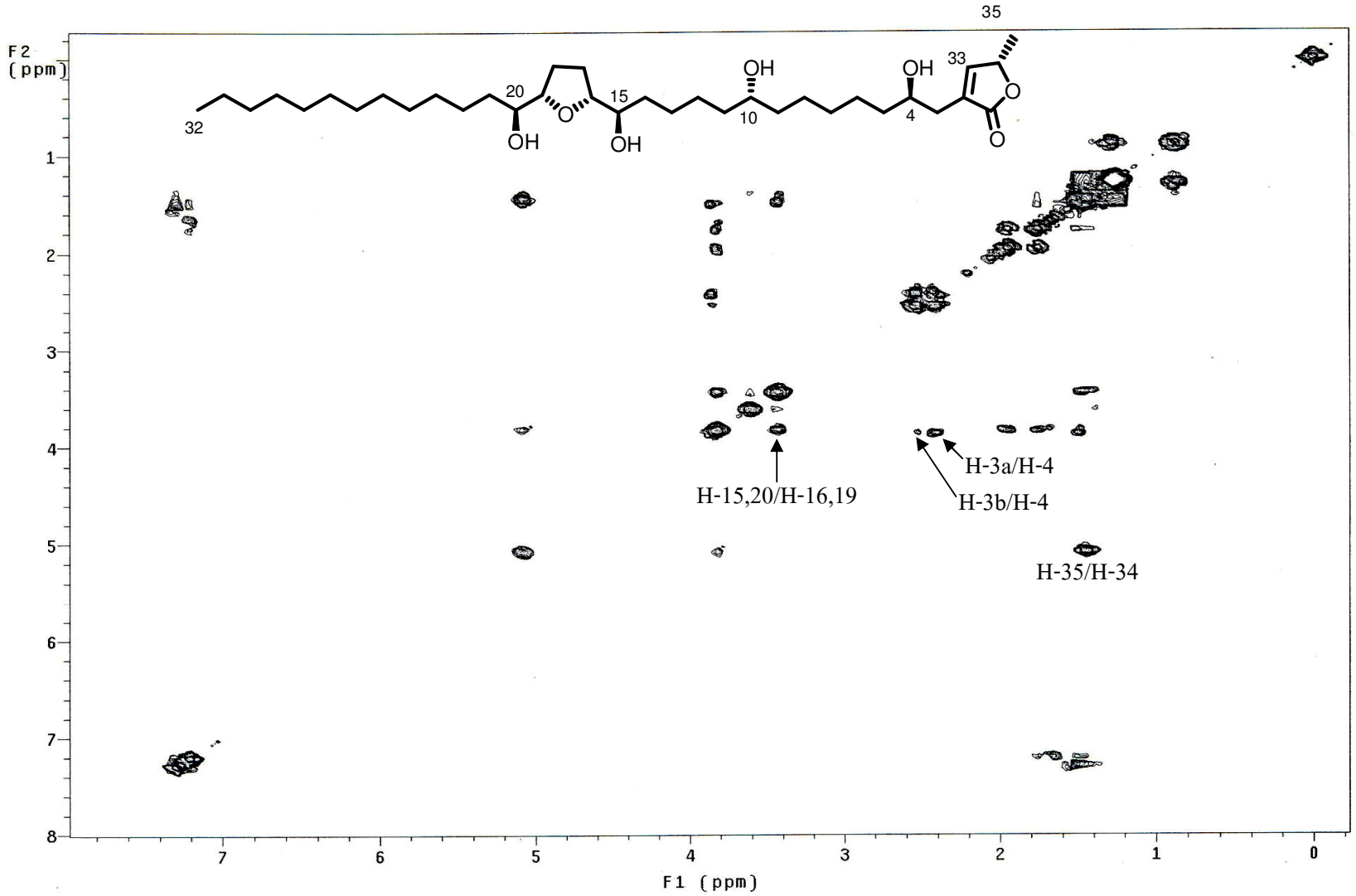
IR spectrum of compound 4



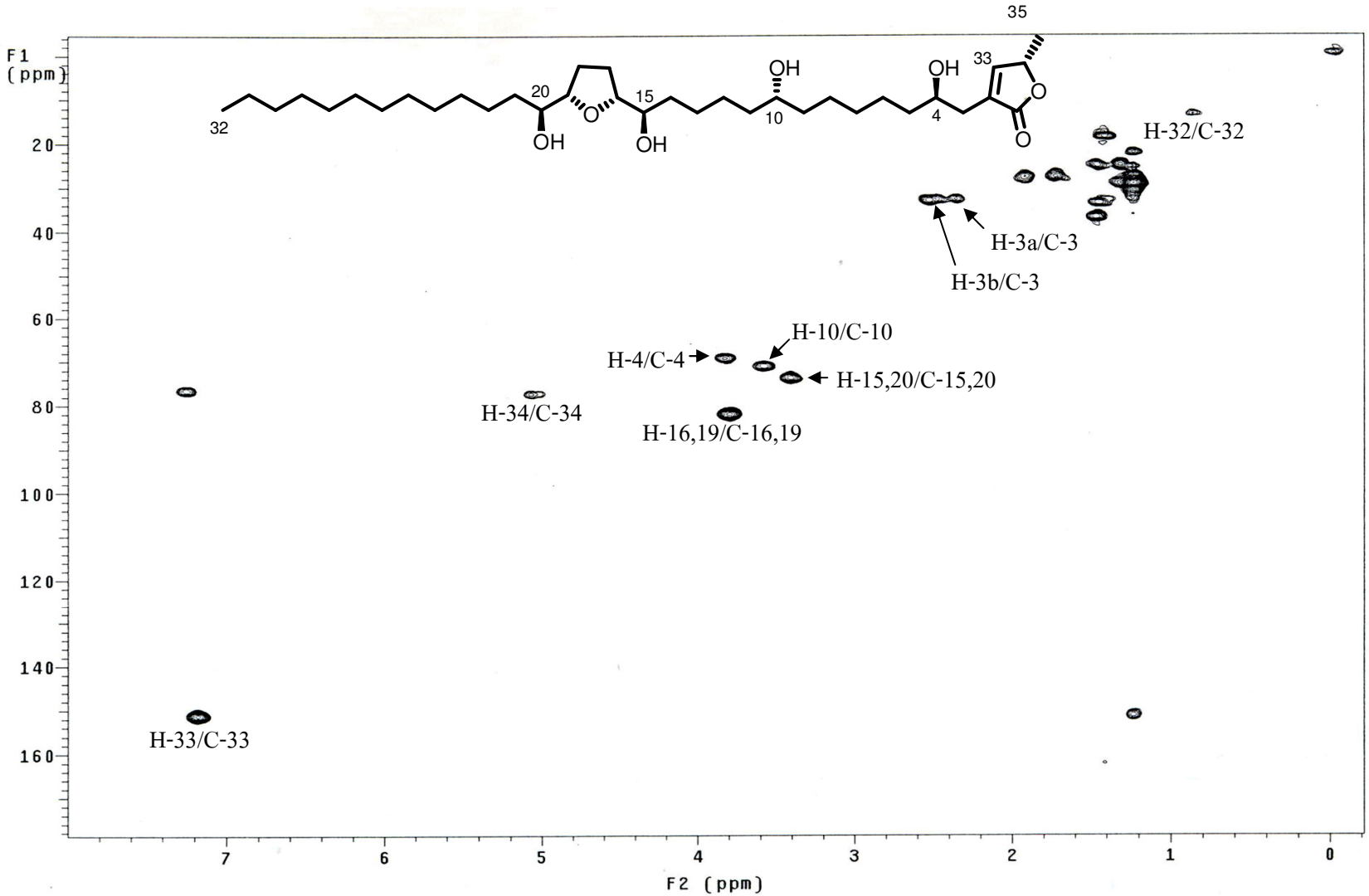
DEPT (90) spectrum of compound 5 in $CDCl_3$



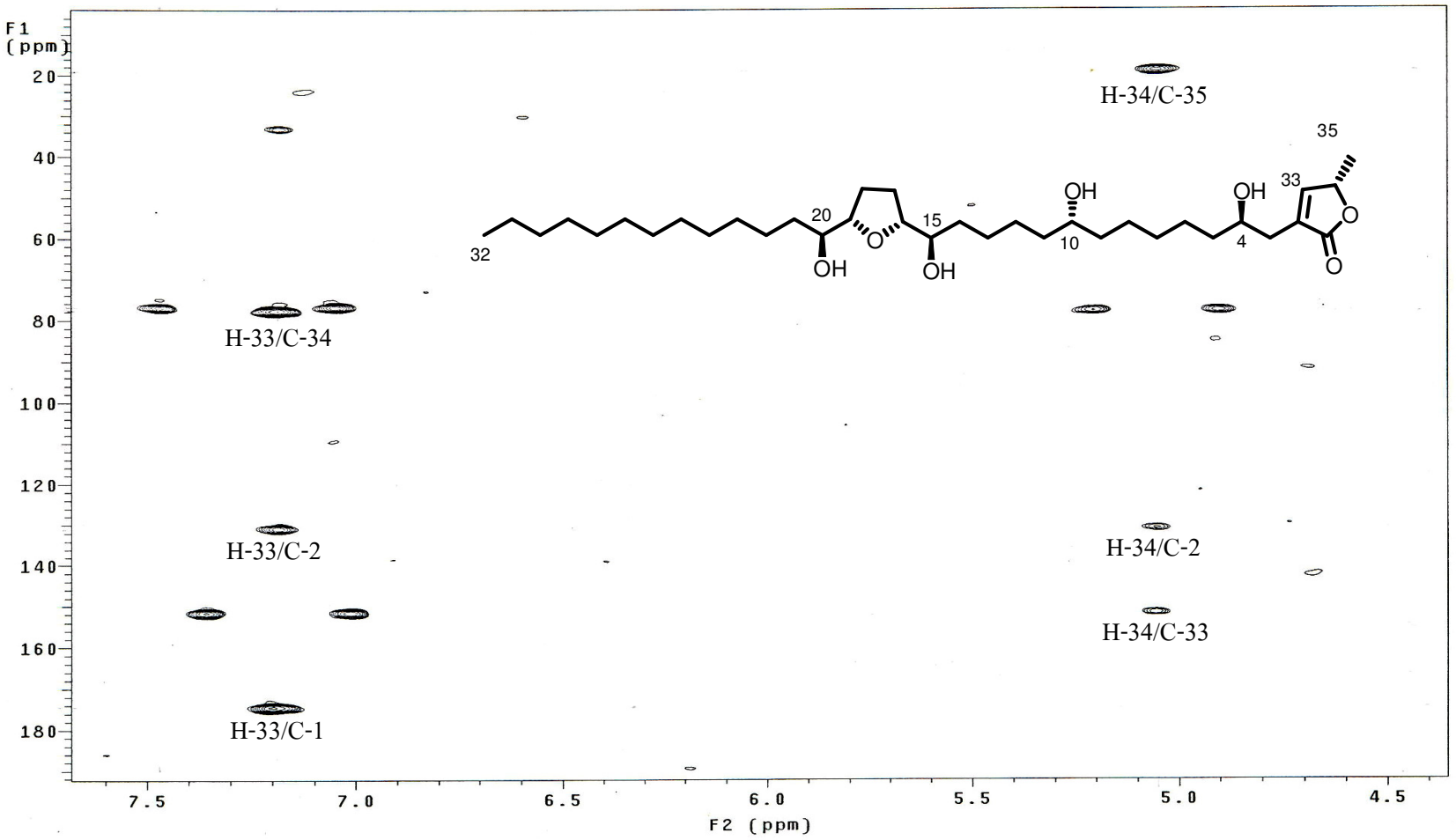
DEPT (135) spectrum of compound 5 in $CDCl_3$



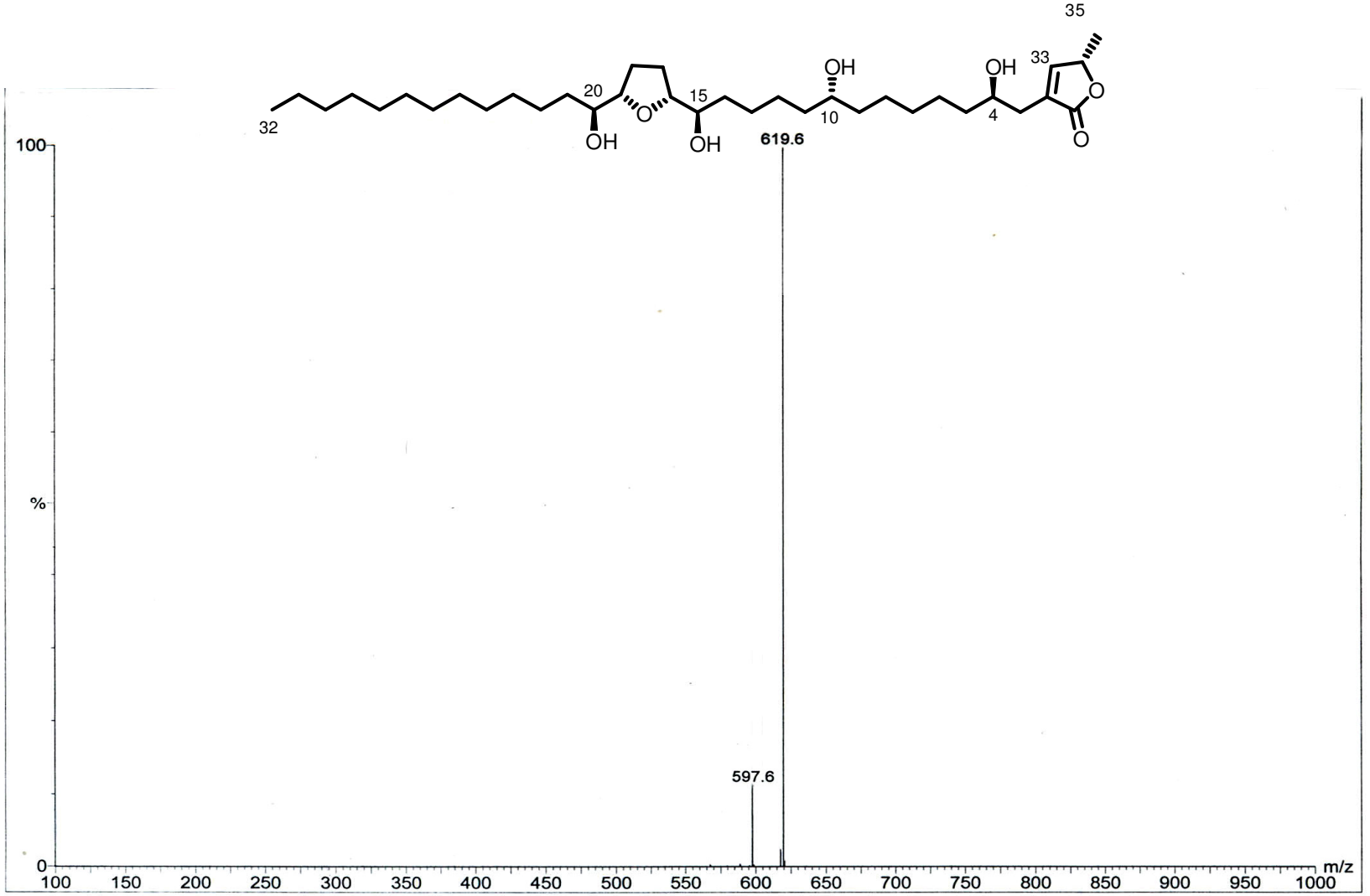
^{1}H-^{1}H COSY spectrum of compound 5 (500 MHz, $CDCl_3$)



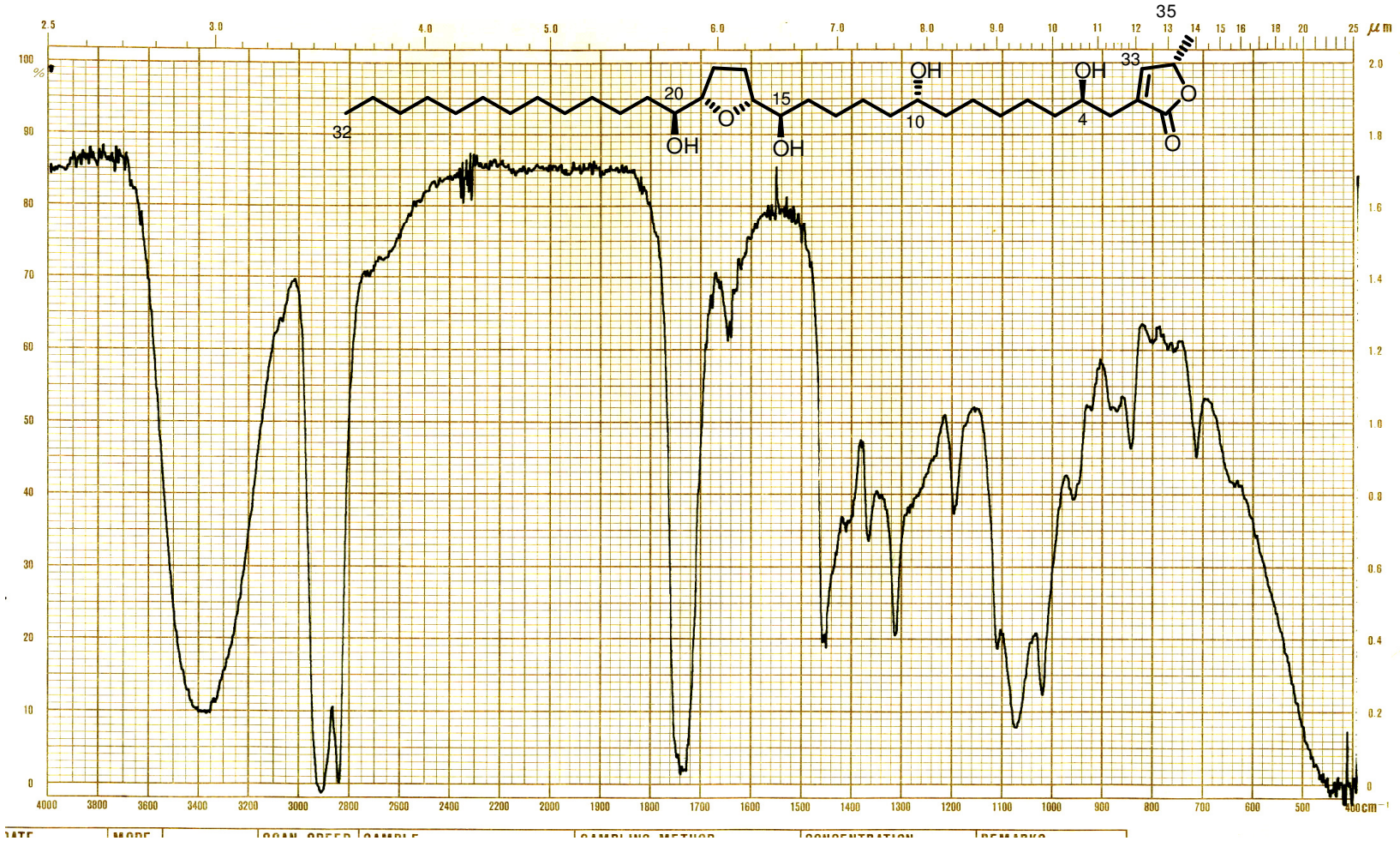
HMQC spectrum of compound 5 (500 MHz, $CDCl_3$)



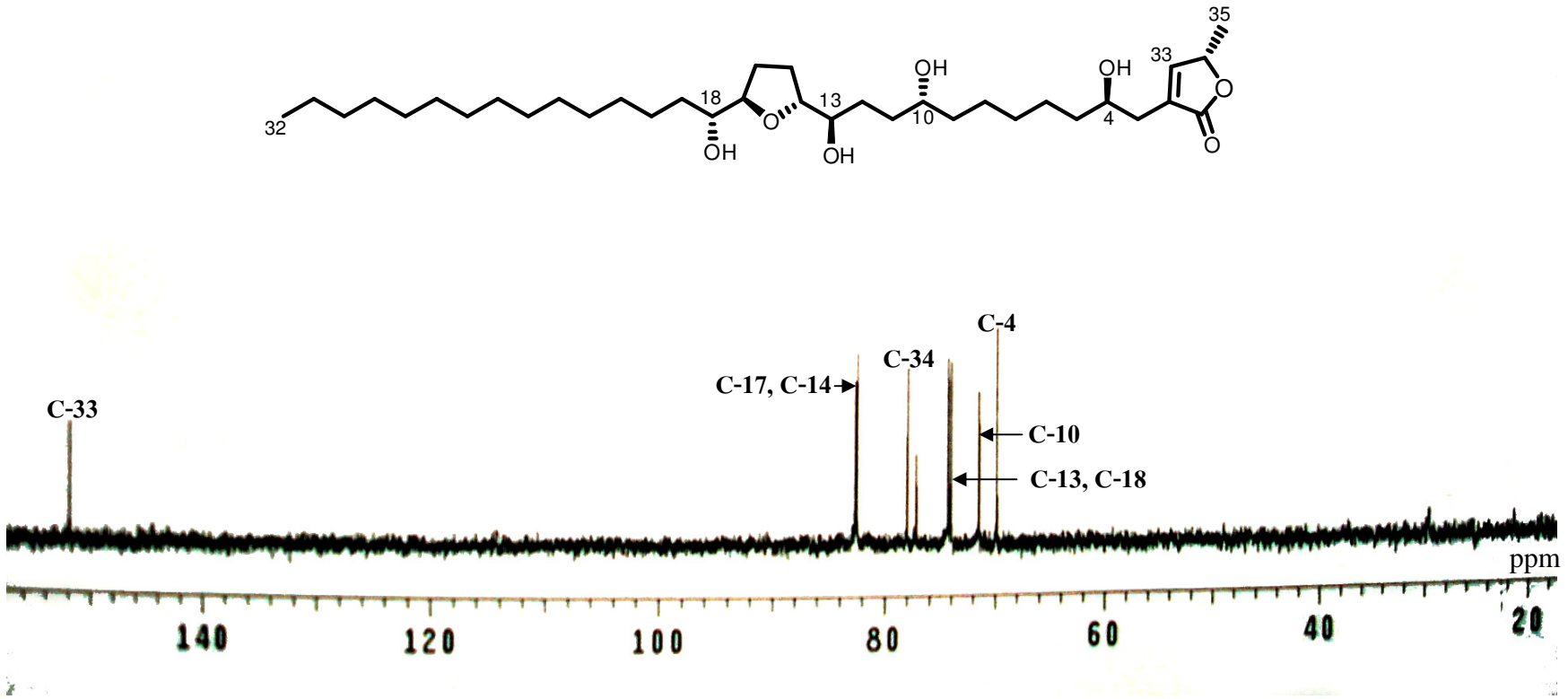
HMBC spectrum of compound 5 (500 MHz, $CDCl_3$)



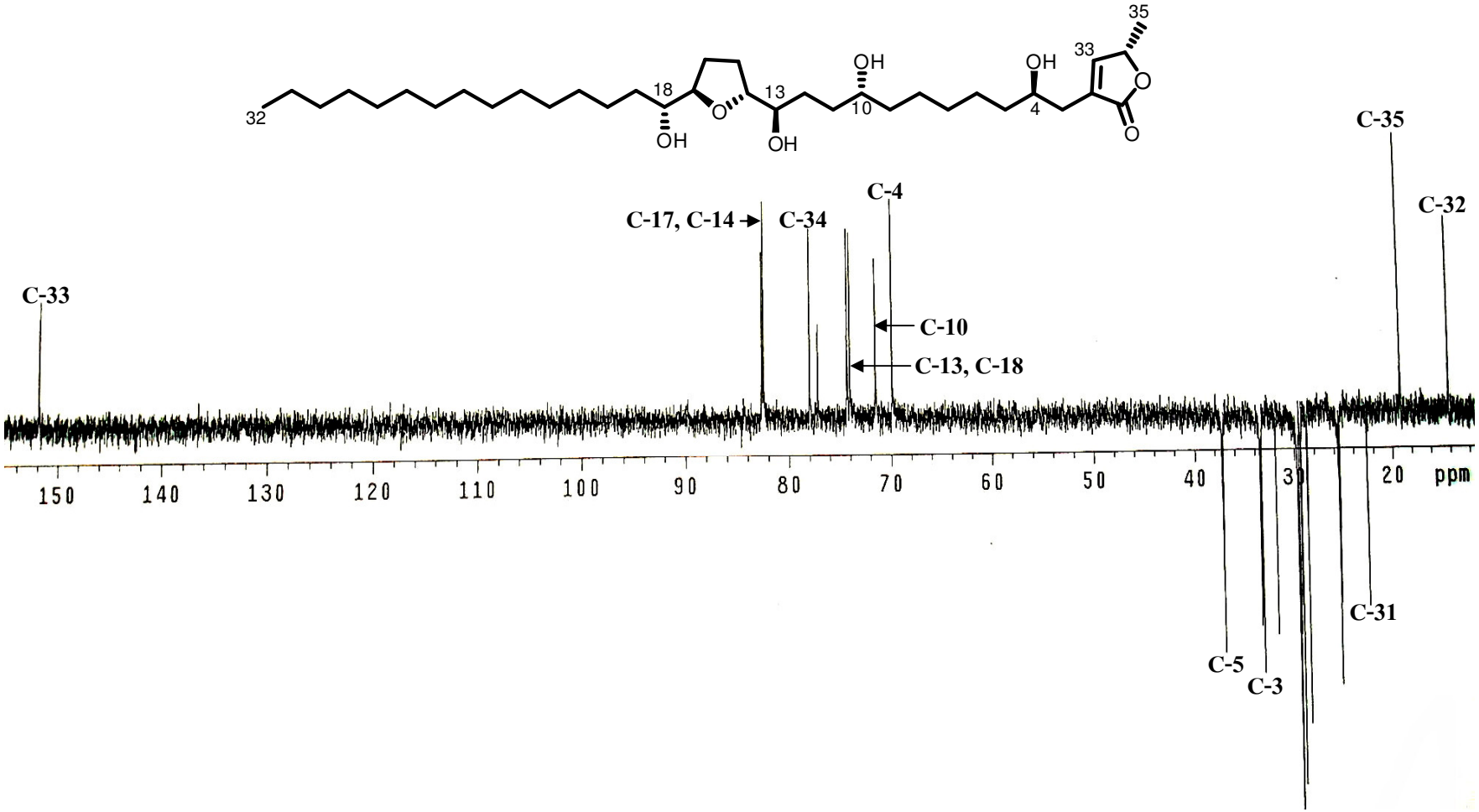
ESIMS spectrum of compound 5



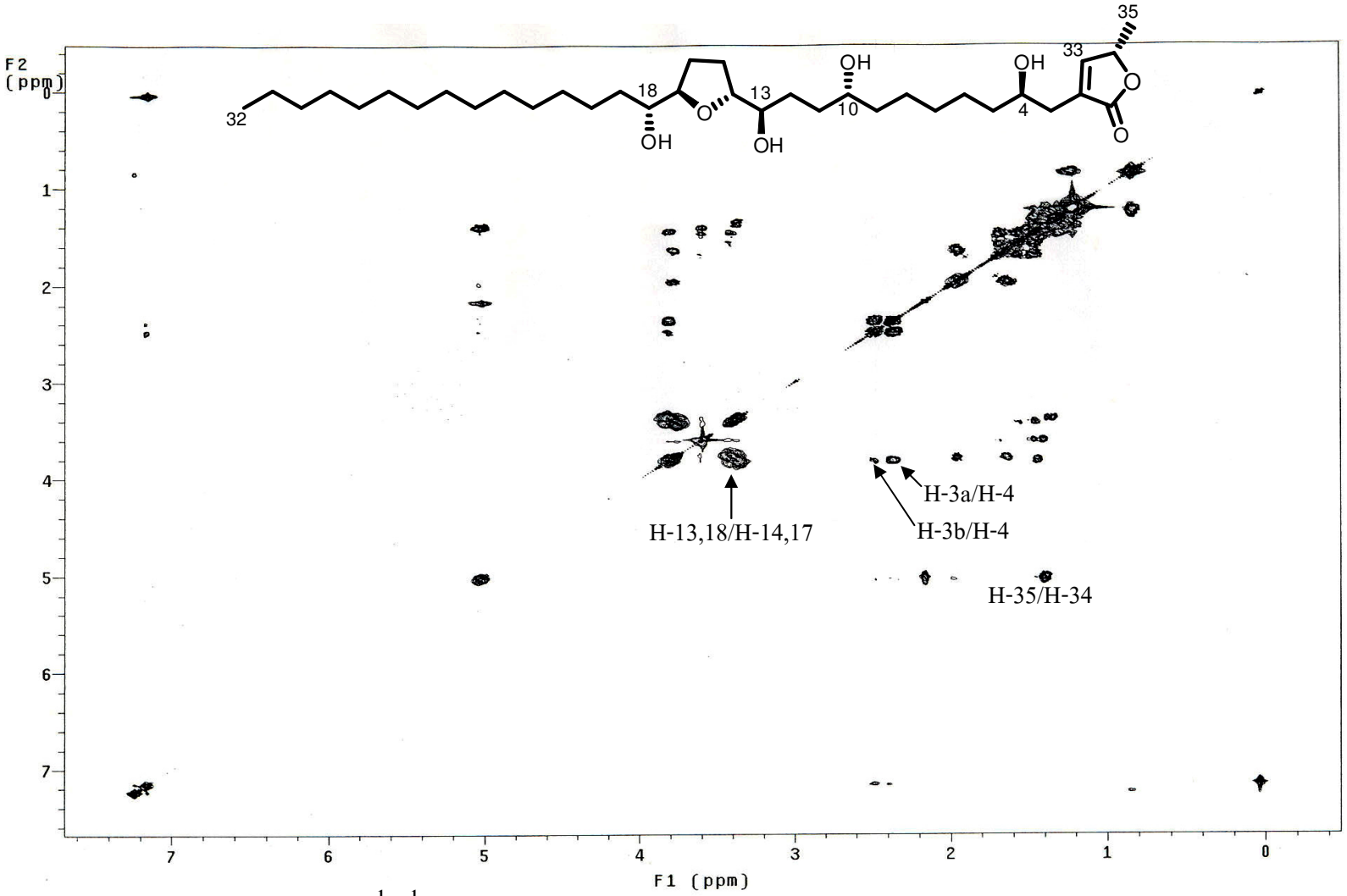
IR spectrum of compound 5



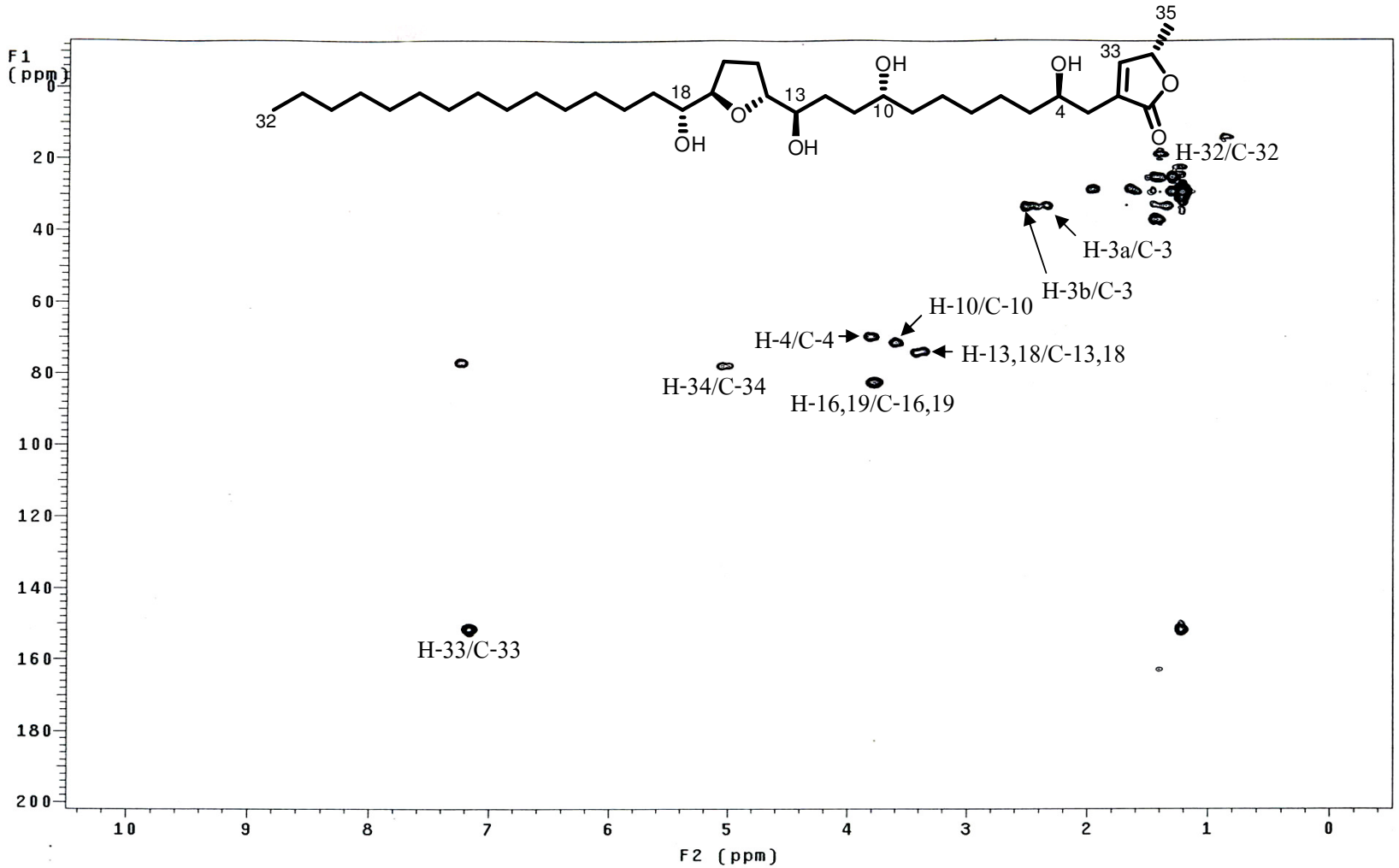
DEPT (90) spectrum of compound 6 in $CDCl_3$



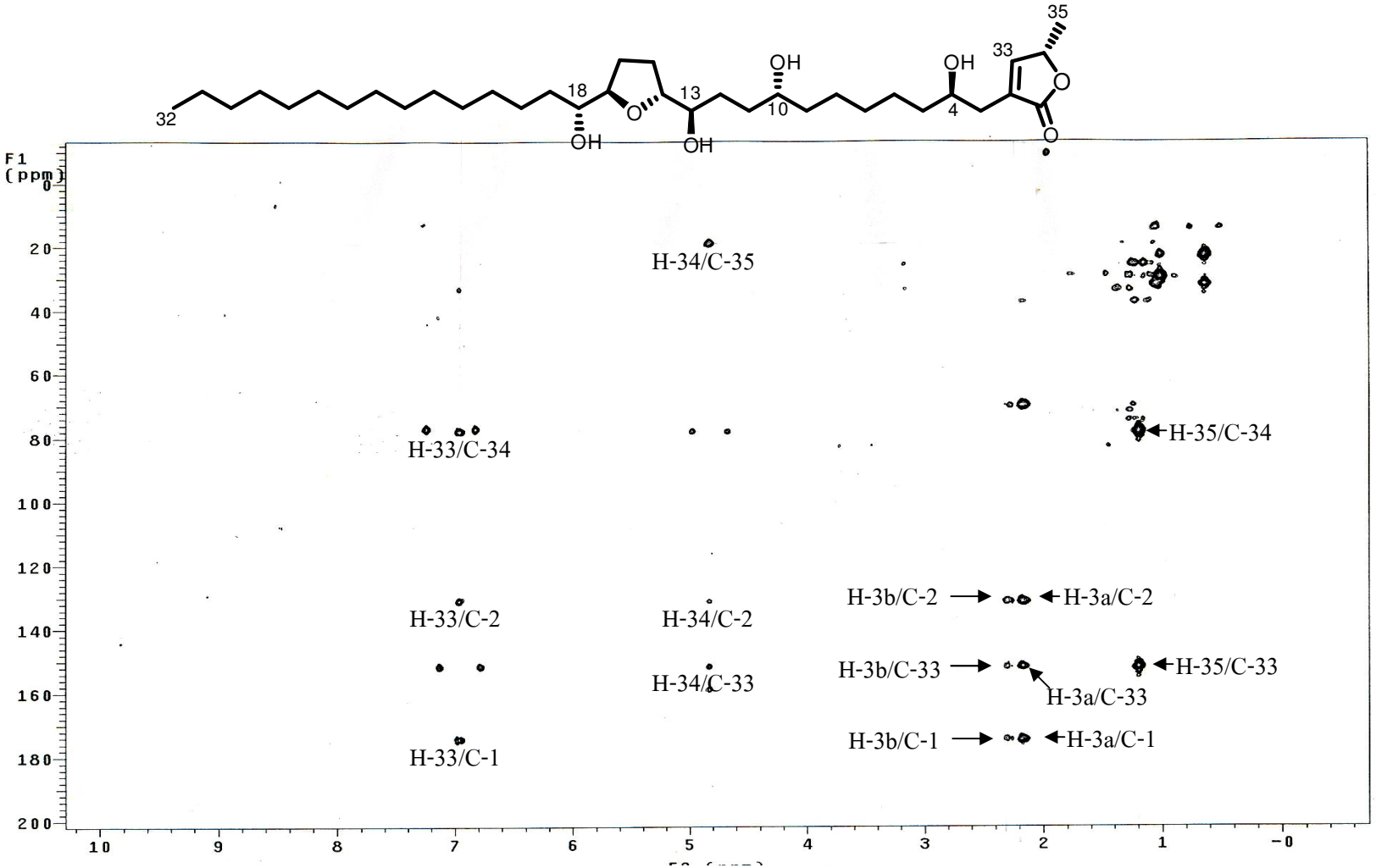
DEPT (135) spectrum of compound 6 in $CDCl_3$



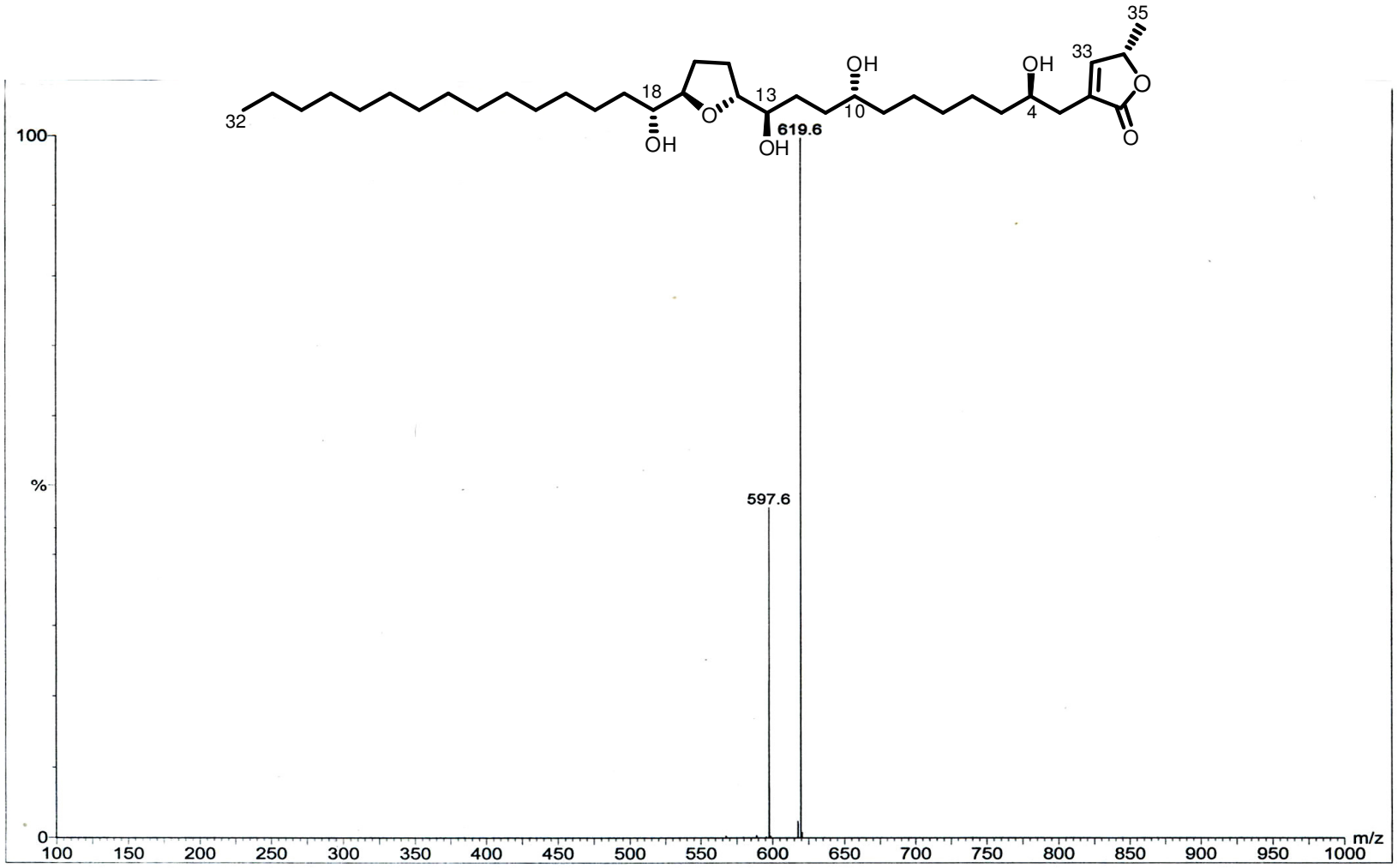
^{1}H-^{1}H COSY spectrum of compound 6 (500 MHz, $CDCl_3$)



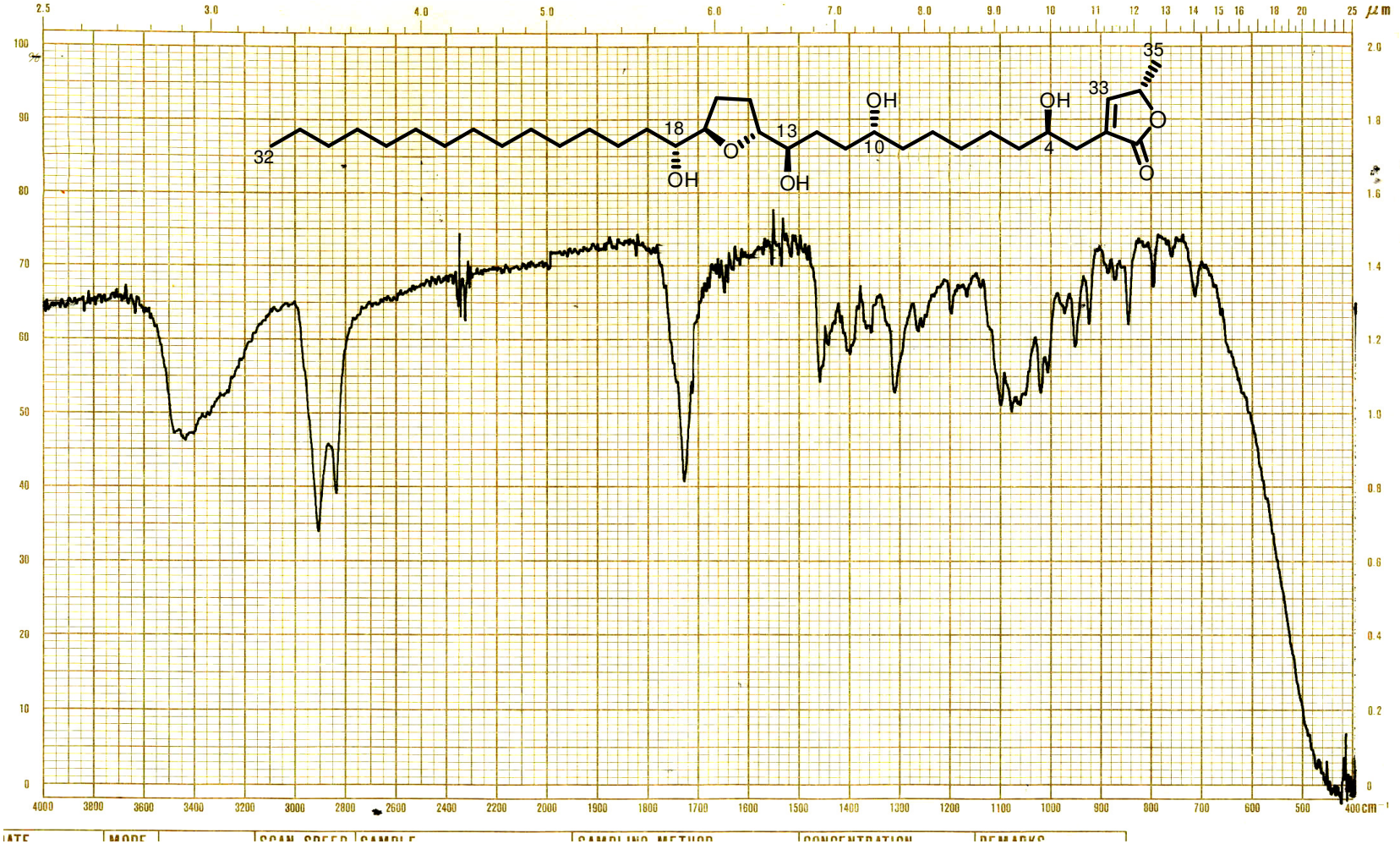
HMQC spectrum of compound 6 (500 MHz, $CDCl_3$)



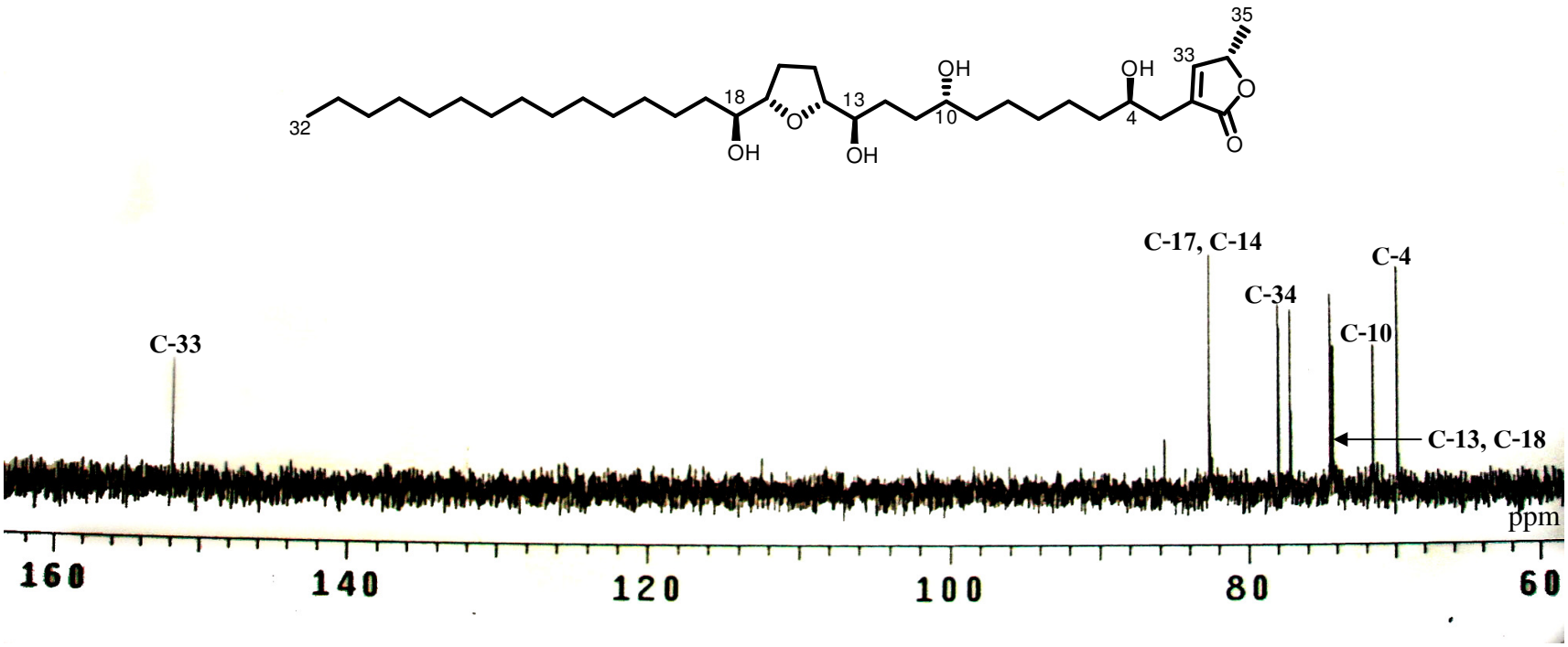
HMBC spectrum of compound 6 (500 MHz, $CDCl_3$)



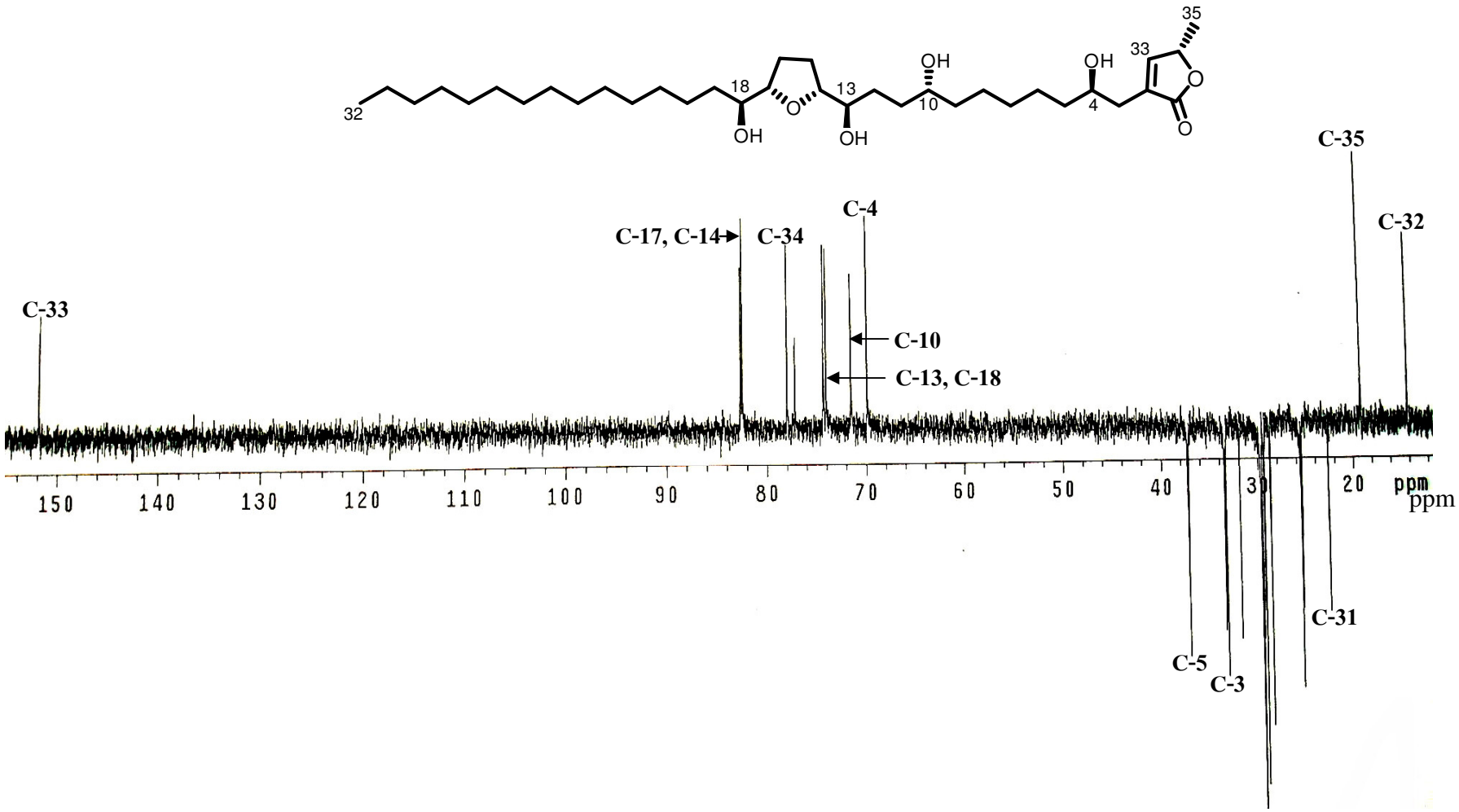
ESIMS spectrum of compound 6



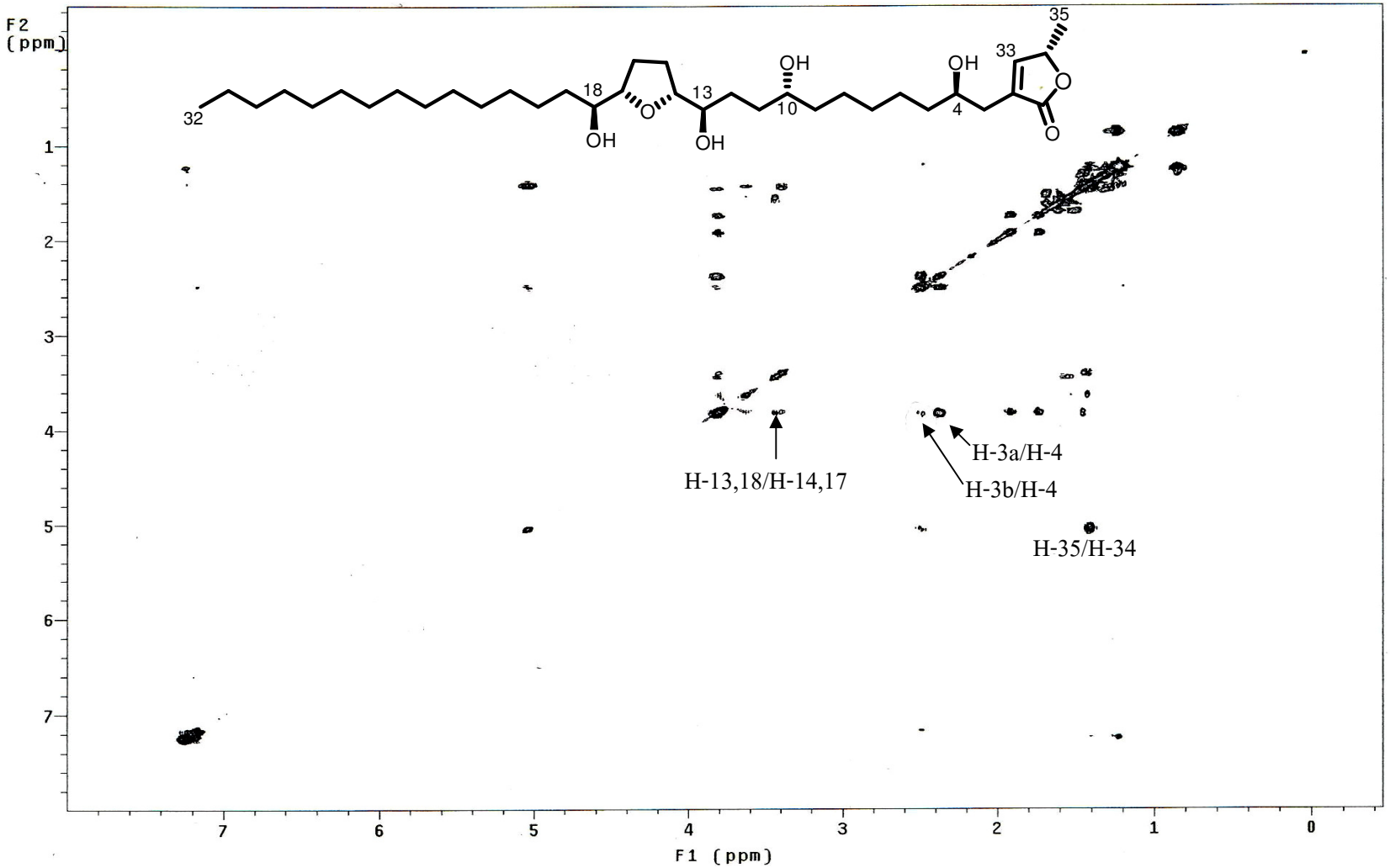
IR spectrum of compound 6



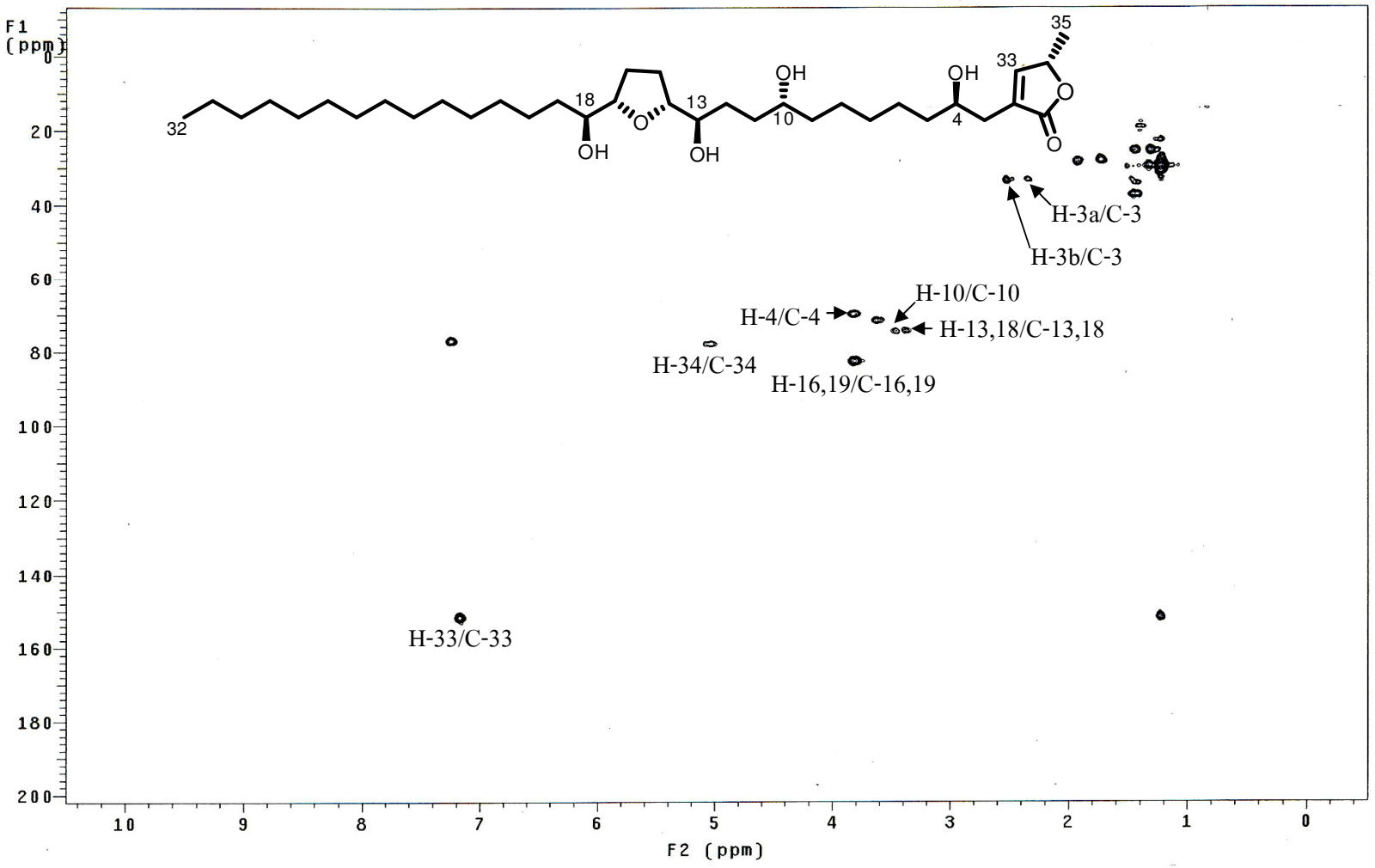
DEPT (90) spectrum of compound 7 in $CDCl_3$



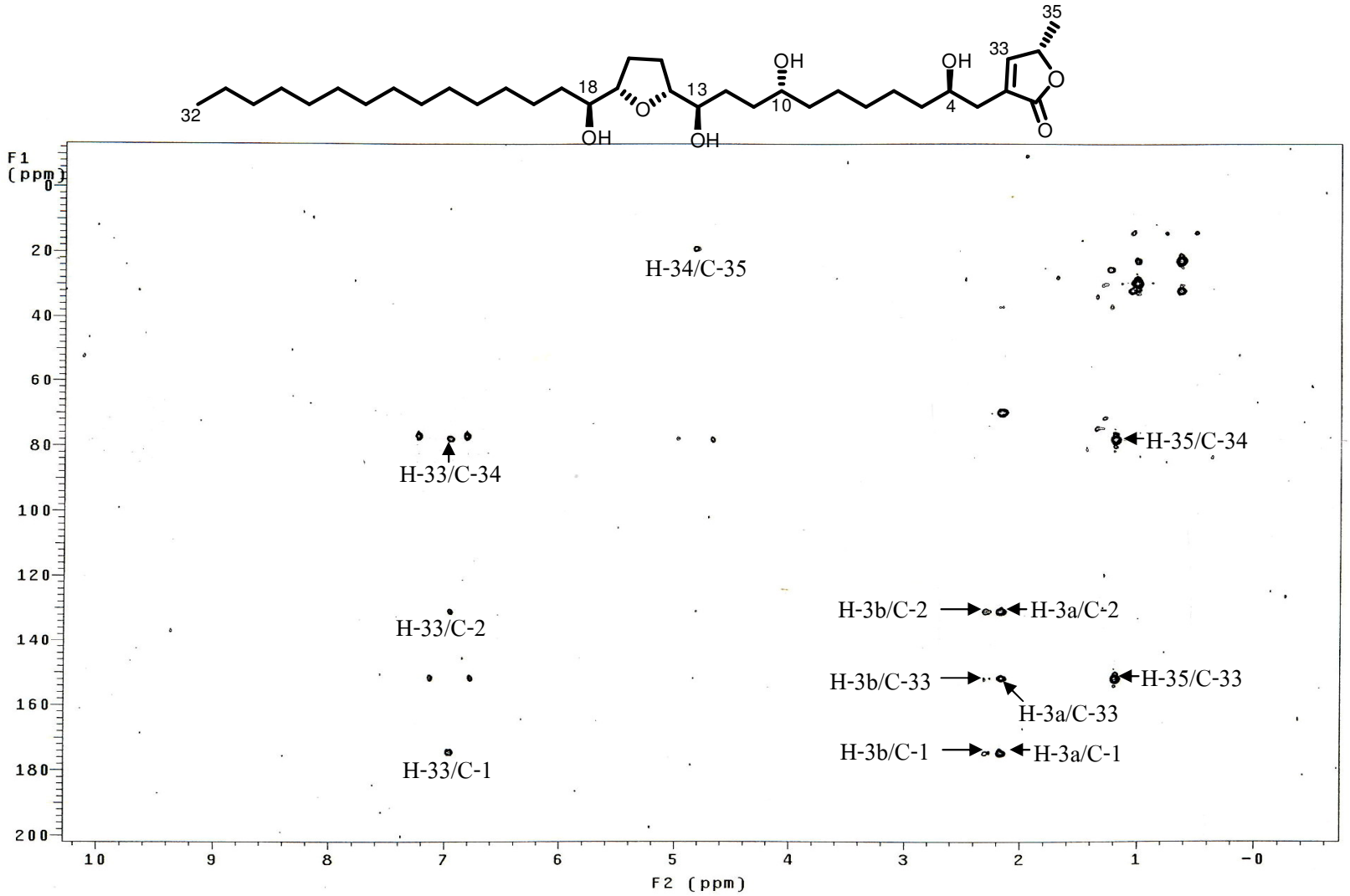
DEPT (135) spectrum of compound 7 in $CDCl_3$



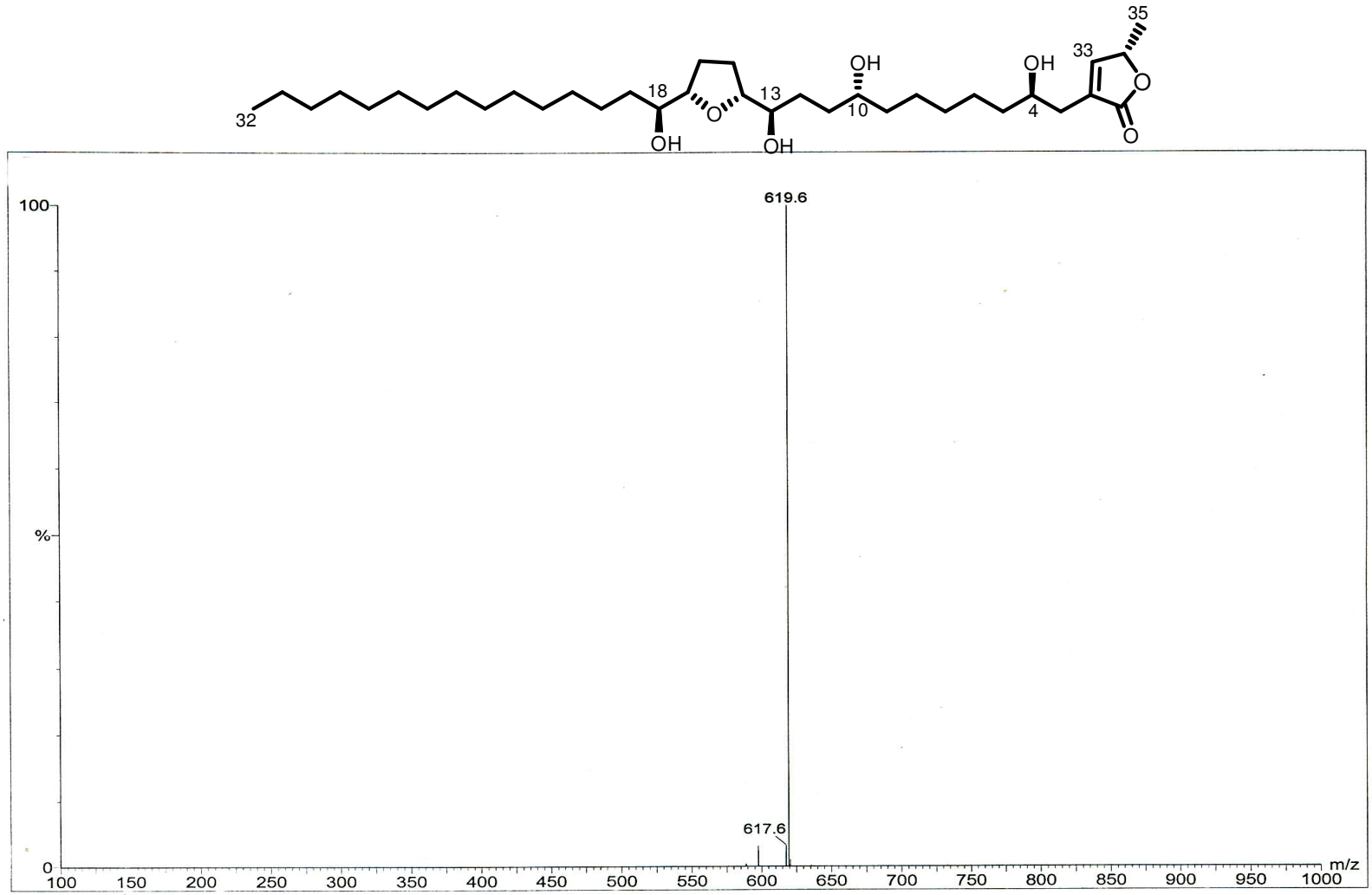
^{1}H-^{1}H COSY spectrum of compound 7 (500 MHz, $CDCl_3$)



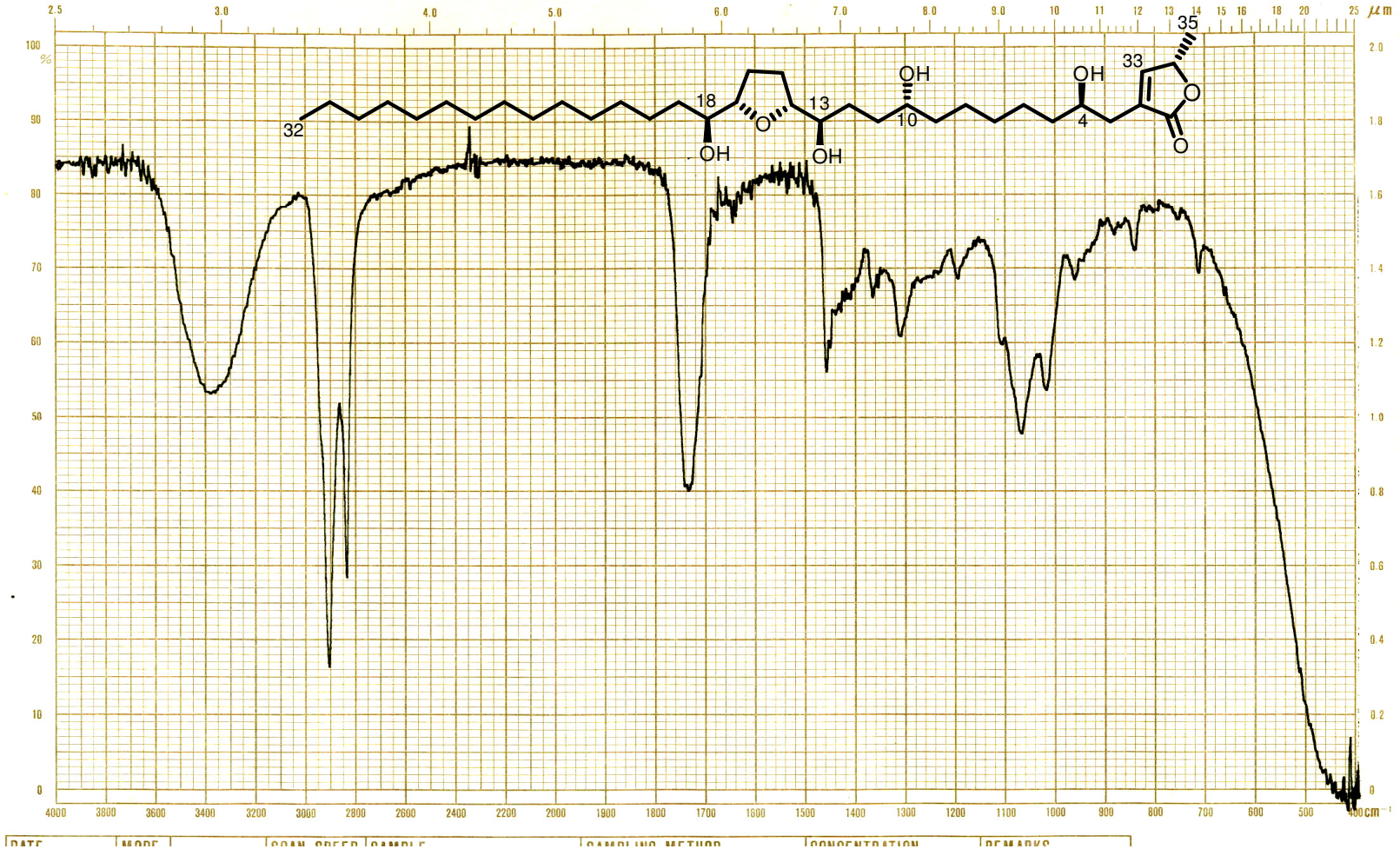
HMQC spectrum of compound 7 (500 MHz, $CDCl_3$)



HMBC spectrum of compound 7 (500 MHz, $CDCl_3$)



ESIMS spectrum of compound 7



IR spectrum of compound 7

VITAE

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Education Attainment

Degree	Name of Institution	Year of Graduation
Bachelor of Pharmacy	Prince of Songkla University	2004

List of Publication and Proceedings

Tantithanaporn, S., Wattanapiromsakul, C., Itharat, A. and Keawpradub, N. 2009. Cytotoxic activity of *Goniothalamus undulatus* Ridl. Proceeding of the 13th National graduate research conference. North-Chiang Mai University, Chiang Mai, Thailand, May 15-16, 2009.