Antitubercular Activity Profiling of Terpenoid Derivatives from the Thai Sponge *Hyrtios* sp.

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ชื่อวิทยานิพนธ์ ระเบียนการออกฤทธิ์ต้านเชื้อวัณโรคของอนุพันธ์เทอร์ปีนอยด์จากฟองน้ำไทยในสกุล *Hyrtios*
ผู้เขียน นายสุนันต์ ใจสมุทร
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บทคัดย่อ

การศึกษาสารประกอบที่แยกได้จากฟองน้ำไทยสกุล *Hyrtios* สามารถแยกสารประกอบชนิดใหม่ 1 ชนิด คือ 12-*epi*-deacetyl-19α-acetoxy-20α-methoxyscalaran (46) และสารที่เคยมีรายงานมาแล้ว 1 ชนิด คือ heteronemin (1) ซึ่งเป็นองค์ประกอบหลักทางเคมีในฟองน้ำชนิดนี้ การดัดแปลงโครงสร้างเพื่อให้ได้อนุพันธ์ของสารประกอบในกลุ่มสกาลารินเพิ่มขึ้นโดยใช้ heteronemin (1) เป็นสารตั้งต้นในการดัดแปลงโครงสร้างใช้ปฏิกิริยาแตกวงแหวนผ่านกระบวนการไฮโดรไลซีส ปฏิกิริยาการปิดวงแหวนหลังกระบวนการรีดักชั่น ปฏิกิริยาออกซิเดชั่นด้วยโครเมต ปฏิกิริยาอะเซทิเลชั่น และปฏิกิริยาแตกโครงสร้างด้วยความร้อน สารประกอบทั้งหมดที่ได้นำมาทดสอบฤทธิ์ต้านเชื้อวัณโรค และความเป็นพิษต่อเซลล์มะเร็ง (MCF-7) พบว่าสารประกอบส่วนใหญ่มีฤทธิ์ต้านเชื้อวัณโรค และฤทธิ์ความเป็นพิษต่อเซลล์มะเร็งในระดับที่ดี เมื่อนำมาวิเคราะห์หาความสัมพันธ์ระหว่างสูตรโครงสร้างกับการออกฤทธิ์ พบว่าเมื่อมีหมู่แทนที่เป็นหมู่อะซีเตท ที่ตำแหน่ง 16 และ 19 จะทำให้อนุพันธ์สกาลารินมีฤทธิ์ต้านวัณโรคระดับที่ดีมาก ส่วนในกรณีความเป็นพิษต่อเซลล์ หมู่แทนที่ที่มีออกซิเจนเป็นส่วนประกอบ ที่คาร์บอนตำแหน่ง 12 มีผลต่อความแรงในการออกฤทธิ์

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Abstract

Chemical investigation of the sponge *Hyrtios* sp. resulted in the isolation and characterization of a new antitubercular sesterterpene, 12-*epi*-deacetyl-19α-acetoxy-20α-methoxyscalaran (**46**), along with the known heteronemin (**1**) as the major component. Chemical derivatizations of heteronemin (**1**) using various transformations, including hydrolytic cleavage, reductive recyclization, chromate oxidation, acetylation, and pyrolysis, also yielded a series of scalarin derivatives. All the scalarins, both from natural products and from chemical transformations, were subjected to antitubercular activity and cytotoxicity (against MCF-7 cell line) determination, to show that all were strongly active in either, or both, assays. The results also suggested that the structure-activity relationship of the antitubercular activity was positively influenced by two acetate functionalities on C-16 and C-19 of the scalarins, whereas the polarity of the oxygenated functional groups on C-12 may affect the cytotoxicity in this model.

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I would like to thank the Biodiversity Research and Training Program (BRT_T65007), Graduate School, Prince of Songkla University, and the Academic Enhancement Program in Pharmaceutical Sciences, Faculty of Pharmaceutical Sciences, for the financial support to conduct this investigation.

Finally, none of this would have been possible without love and encouragement of my friends. I thank them all for their understanding during all of the times when I could not be with them. Their steady love, indeed, supported me.

Sunan Jaisamut

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LIST OF ABBREVIATIONS AND SYMBOLS

$[\alpha]_D$	specific rotation
δ	chemical shift in ppm
ε	molar extinction coefficient
λ_{max}	maximum wavelength
ν_{max}	wave number
2D NMR	two-dimensional nuclear magnetic resonance
Ac	acetyl
br	broad (for NMR signals)
c	concentration
CFU	colony forming units
CoMFA	comparative molecular field analysis
CoMSIA	comparative molecular similarity indices analysis
COSY	correlation spectroscopy
d	doublet (for NMR signals)
DEPT	distortionless enhancement by polarization transfer
DMSO	dimethylsulfoxide
EIMS	electron-impact mass spectroscopy mass spectroscopy
ESIMS	electro-sprayed ionization mass spectroscopy
HMBC	heteronuclear multiple bond correlation
HMQC	heteronuclear multiple-quantum coherence
HREIMS	high-resolution electron-impact mass spectroscopy
HRESIMS	high-resolution electro-sprayed ionization mass spectroscopy
IC_{50}	inhibitory concentration at 50% of tested subject
IR	infrared
J	coupling constant
LAH	lithiumaluminum hydride
m	multiplet (for NMR signals)
MABA	microplate alarma blue assay
Me	methyl
MIC	minimum inhibitory concentration
MS	mass spectroscopy

LIST OF ABBREVIATIONS AND SYMBOLS (continued)

m/z	mass-over-charge ratio
nOe	nuclear Overhauser effect
NMR	nuclear magnetic resonance
PCC	pyridinium chlorochromate
ppm	part per million
q	quartet (for NMR signals)
s	singlet (for NMR signals)
SD	standard deviation
SRB	sulphorhodamine B
t	triplet (for NMR signals)
THF	tetrahydrofuran
UV-VIS	ultraviolet-visible

CHAPTER 1

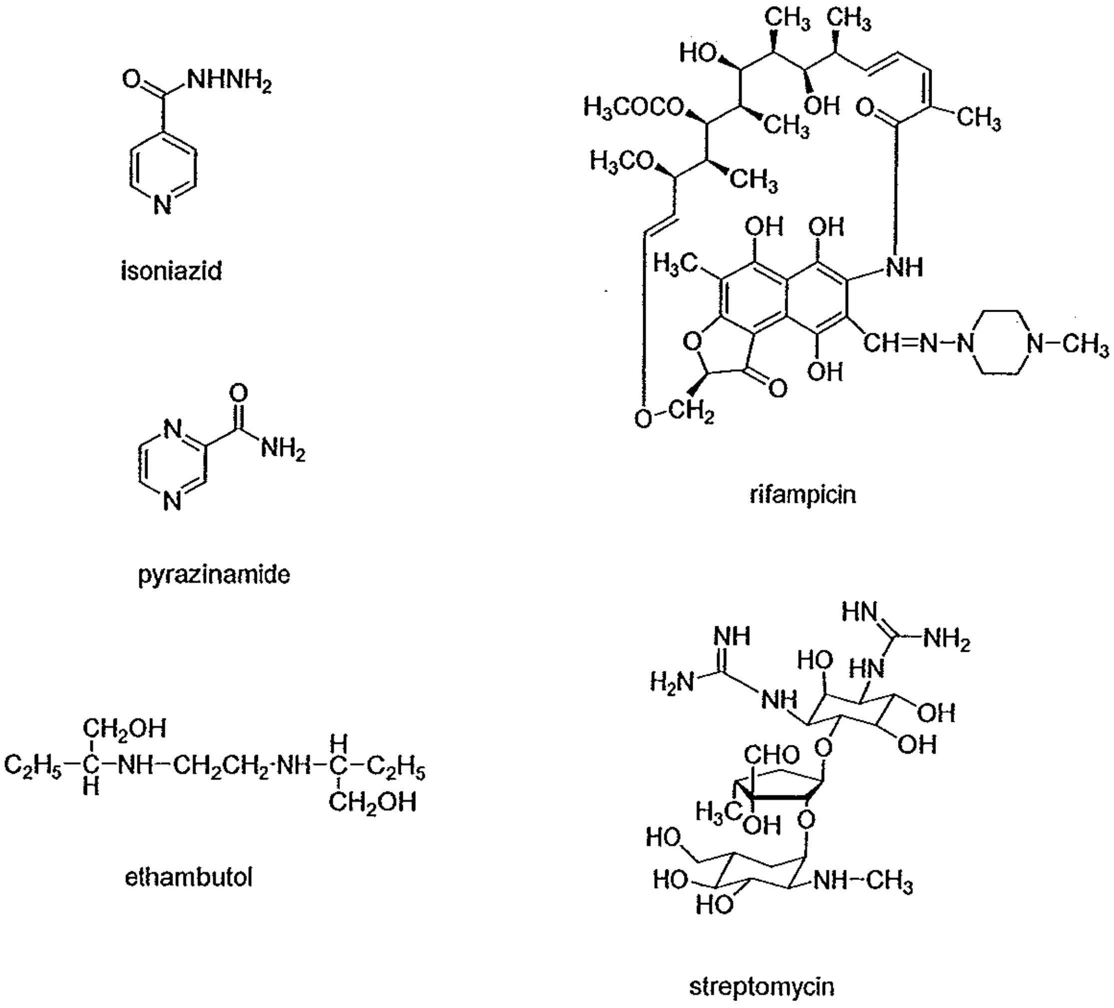
INTRODUCTION

1.1 General introduction: Tuberculosis

Tuberculosis (TB), caused by *Mycobacterium tuberculosis,* is among the most vicious infectious diseases that has plaqued human beings since the earliest of time. According to the recent report by the World Health Organization (WHO), a total of 8 million world's population were infected by *M. tuberculosis*, and as many as 1.5 million deaths were reported associated with tuberculosis (WHO, 2007).

Despite the efforts both by local governments worldwide and by WHO to control the spreading of the disease, the battle with tuberculosis seems to be the one that men will always keep losing grounds. In 1993, WHO declared tuberculosis as a global emergency, and has put high efforts both on surveillance and on treatments of the disease. However, with one vaccine (BCG) and limited number of effective antibacterial agents as the only measures, such efforts are never adequate. The association of tuberculosis with poverty, the need of long-term treatment, and the latent nature of the disease are all combined to worsen the epidemic situation. Also, as one of the most common infections in immuno-compromised patients, the coinciding recurrent of tuberculosis outbreak since the 1990's with the pandemic of HIV/AIDS is remarkably evident.

Currently, first-line drugs used in the treatment of TB include isoniazid, rifampicin, pyrazinamide, ethambutol, and streptomycin (Figure 1) (Sensi and Grassi, 1996). Short-course regimens using initially at least three first-line drugs are effective, and combination therapy has been well documented to reduce the emergence of *M. tuberculosis* strains that are resistant to individual agents. As mentioned earlier, the poverty as a major problem faced in TB control leads to the lack of early diagnosis and short in drug supply, as well as to patient's failure to complete their course of drugs. As a result, multi-drug resistant (MDR) strains of *M. tuberculosis*, defined as strains with the resistance to at least isoniazid and rifampicin, have been emerged (Duncan, 1997; Inderlied, 1999). There were approximately 3.2% of newly estimated TB cases world-wild that were MDR-TB in 2000 (Espinal, 2003). Second-line drugs, including ethionamide, cycloserine, kanamycin, capreomycin, amikacin, para-aminosalicylic acid and thiacetazone, which are less efficacious and/or more toxic than first-line ones, are obligated in such cases (Glassroth, 2001).



isoniazid

rifampicin

pyrazinamide

ethambutol

streptomycin

Figure 1 First-line drugs for tuberculosis

Whereas new technologies that lead to new drug discoveries including computer-aided drug design, molecular biology, and gene therapy, have been developed extensively in such way that a direct- and target-oriented medicine is no longer mere theoretical prospects, such developments seems not to be employed to a satisfying level in the area of antitubercular drugs. In fact, over the past 30 years, no new antitubercular agents with new mechanisms have been developed. There have been a number of practical obstacles in developing new antituberculosis agents. Among these is the lack of economic incentive due to the predominance of the disease in the developing world. The very slow growth and highly contagious nature of *M. tuberculosis* have also discouraged the drug discovery effort (Cantrell *et al.*, 2001). Yet new-drug discovery with new and different modes of actions is among urgent needs to control the spread of drug-resistant strains as well as to lower the mortality rate of MDR-TB.

The oceans, covering more than 70% of the earth's surface, have been long known as the ecological habitats with a highly unique and wide-ranged biodiversity. The uniqueness that earns marine biota the excellent candidacy as the producers of novel biologically active agents is possibly due to the physical and chemical differences between marine and terrestrial environments, such as the great density of the sea water, the reduced light permeation thus allowing photosynthesis only in a narrow surface zone, and the skeletons of the biosynthetic starting materials, which are protein-dominated (as compared to the carbohydrate-dominance in terrestrial plants). Besides these properties, the food chain in the marine environment is also far more complex than that in the terrestrial counterpart. The abundance of filter-feeding sessile organisms, which serve as excellent substrata for epibionts and symbionts, extends marine ecosystems to become more complex communities that are either absent or rare in terrestrial ecosystems (Scheuer, 1990). Furthermore, ecological stresses, including predation, competition for space, and fouling on the surface, lead to the evolution of various forms of defense mechanisms including the accumulation of unique secondary metabolites with various biological activities toward either predators or preys (König *et al.*, 1994). Altogether, these have proved to be beneficial to the discovery of drugs with greater efficacy and specificity for the treatment of several diseases than those currently used in clinic.

Among the marine organisms, sponges are the first marine invertebrate group that were studied for their secondary metabolites (Bergquist, 1978). To date, sponges have yielded a great number of novel bioactive compounds (Faulkner, 1995). The sponges, belonging to the phylum Porifera, are the most primitive group of multicellular animals existing as far back as Precambriam periods or approximately 600-700 million years ago (Allen, 1996). They are sedentary and feed on their food by filtering the microplanktons from sea water passing through the small holes on their bodies (Bergquist, 1978). To survive for such a long period of time, sponges have to fight off even more sophisticated predators and to compete for space by producing distasteful or otherwise deterrent chemicals. Interestingly, these chemicals are intrinsically bioactive and are therefore the compounds that researchers seek today as potential medicines (Faulkner, 1995). Furthermore, the filtration of sea water makes sponges a great reservoirs of the metabolites from marine microorganisms. Besides, the colonies of sponges also serve as symbiotic systems, in which large number of epibionts such as bacteria and other microorganisms reside in a unique association. Consequently, unusual metabolites that were produced by the microorganisms can be found in sponges (König and Wright, 1996). It is thus not surprising that many marine natural products from sponges are highly active in many pharmacological assays.

Whereas most compounds from marine sponges, or even from other marine invertebrates, have been primarily reported as cytotoxic agents, a significant number of marine natural products are also active in other biological models, including anti-infectious activity. Of particular interest in this review, a handful of antitubercular agents of marine origins have readily been reported, and are listed in Table 1.

Table 1 Marine natural products as antitubercular agents

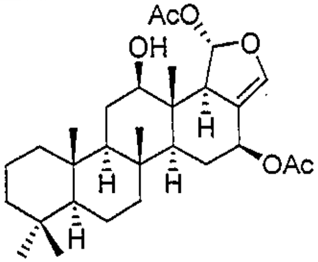
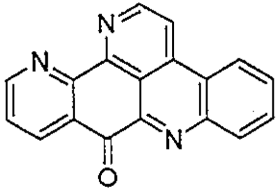
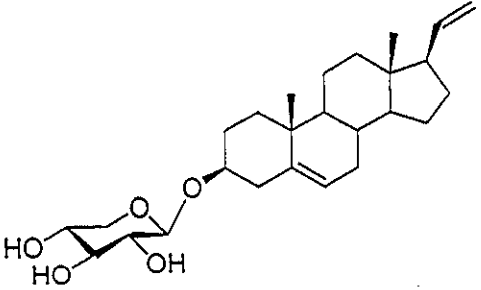
Sources	Names	Structures	Antitubercular activity	References
Hyrtios sp.	Heteronemin (1)	 1	MIC = 6.25 μg/mL	Kazlauskas *et al.*, 1976
Didemnum sp.	Ascididemin (2)	 2	MIC = 0.25 μg/mL	Kobayashi *et al.*, 1988; Chung *et al.*, 1995
Eunicea pinta	Pregnene saponin (3)	 3	MIC = 6.25 μg/mL	Iguchi *et al.*, 1989; Shi *et al.*, 2002

Table 1 (cont.)

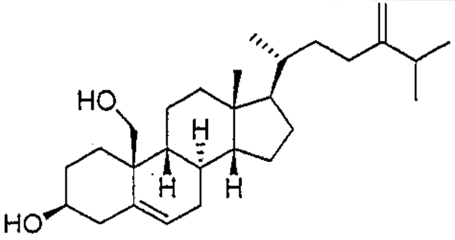
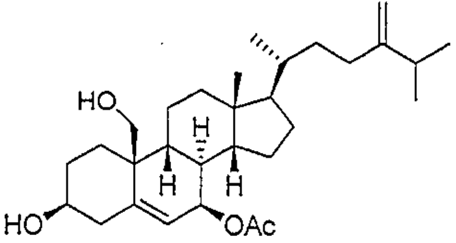
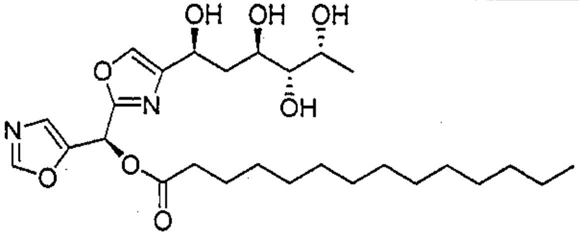
Sources	Names	Structures	Antitubercular activity	References
Nephthea sp.	Litosterol (4)	 **4**	MIC = 3.13 μg/mL	Liu *et al.*, 1992
Nephthea sp.	Nephalsterol-C (5)	 **5**	MIC = 12.5 μg/mL	Liu *et al.*, 1992
Jaspis sp.	Bengazole A (6)	 **6**	MIC = 0.5 μg/disk	Molinski *et al.*, 1993

Table 1 (cont.)

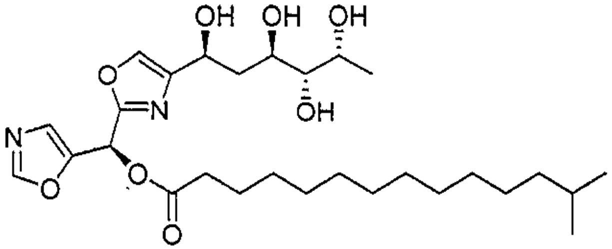
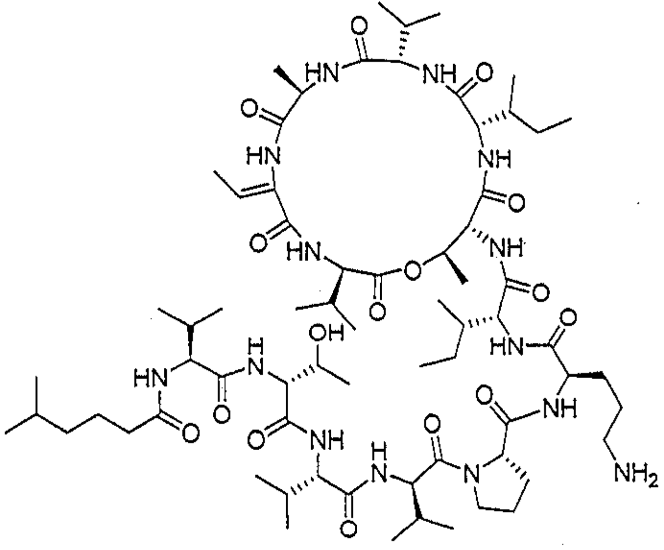
Sources	Names	Structures	Antitubercular activity	References
Jaspis sp.	Bengazole B (7)	 7	MIC = 0.5 μg/disk	Molinski *et al.*, 1993
Elysia rufescens	Kahalalide F (8)	 8	67% Inhibition at 12 mg/mL	Hamann *et al.*, 1996

Table 1 (cont.)

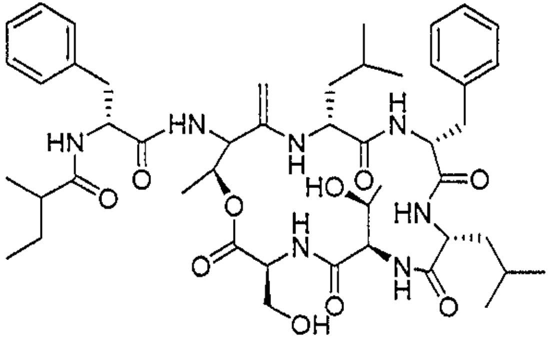
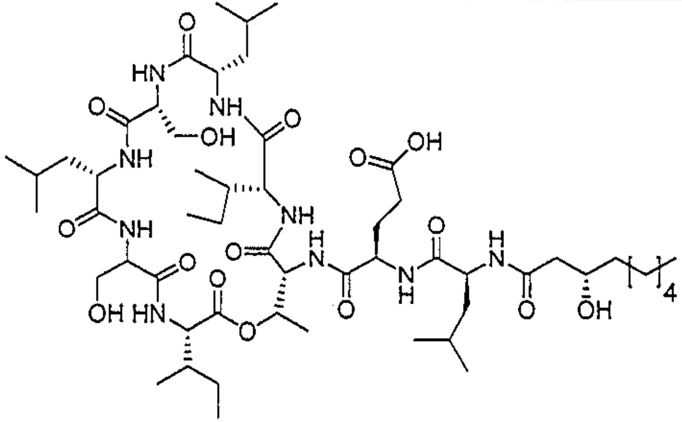
Sources	Names	Structures	Antitubercular activity	References
Elysia rufescens	Kahalalide A (9)	 **9**	83% Inhibition at 12 mg/mL	Hamann *et al.*, 1993
Pseudomonas sp.	Massetolide A (10)	 **10**	MIC = 5 μg/mL	Gerard *et al.*, 1997

Table 1 (cont.)

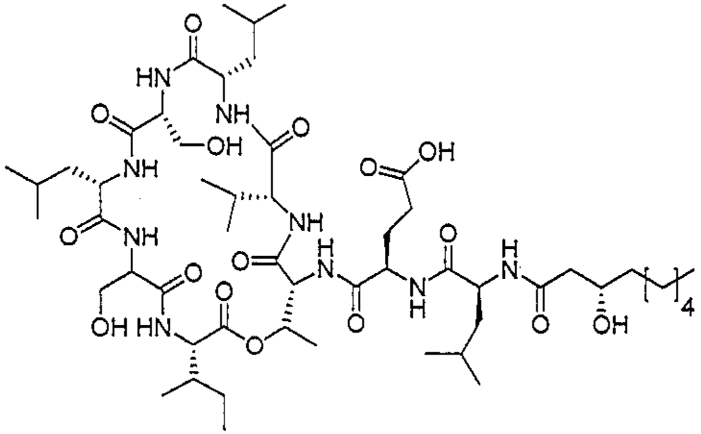
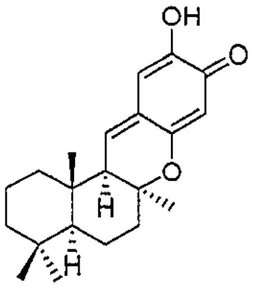
Sources	Names	Structures	Antitubercular activity	References
Pseudomonas sp.	Viscosin (**11**)	 **11**	MIC = 10 μg/mL	Gerard *et al.*, 1997
Hyrtios sp.	Puupehenone (**12**)	 **12**	IC_{50} = 2 μg/mL	Zjawiony *et al.*, 1998; El Sayed *et al.*, 2000

Table 1 (cont.)

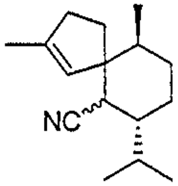
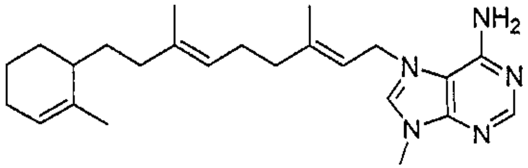
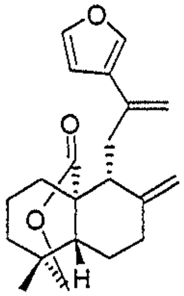
Sources	Names	Structures	Antitubercular activity	References
Acanthella klethra	Axisonitrile-3 (13)	 13	MIC = 2.0 μg/mL	König *et al.*, 2000
Agelas sp.	Agelasine F (14)	 14	MIC = 3.13 μg/mL	Mangalindan *et al.*, 2000
Potamogeton malaianus	Potamogetonin (15)	 15	MIC = 100 μg/mL	Kittakoop *et al.*, 2001

Table 1 (cont.)

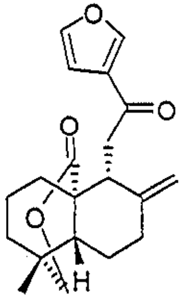
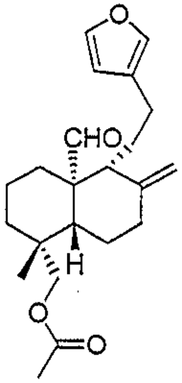
Sources	Names	Structures	Antitubercular activity	References
Potamogeton malaianus	Potamogetonin analog (16)	 **16**	MIC = 100 μg/mL	Kittakoop *et al.*, 2001
Potamogeton malaianus	Potamogetonyde (17)	 **17**	MIC = 100 μg/mL	Kittakoop *et al.*, 2001

Table 1 (cont.)

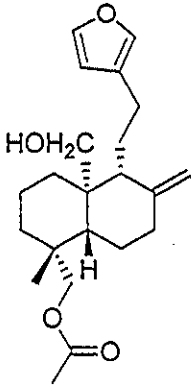
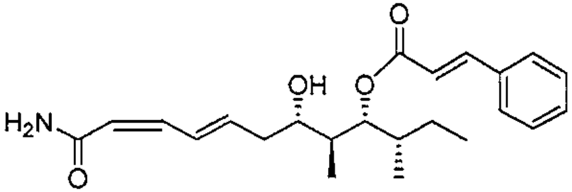
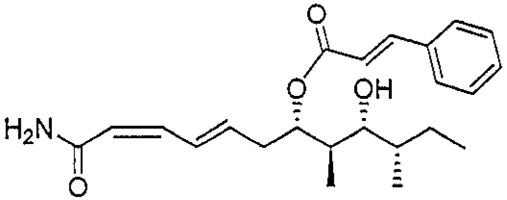
Sources	Names	Structures	Antitubercular activity	References
Potamogeton malaianus	Potamogetonol (18)	 **18**	MIC = 50 μg/mL	Kittakoop *et al.*, 2001
Bacillus laterosorus	Basiliskamides A (19)	 **19**	MIC = 1 μg/mL	Barsby *et al.*, 2002
Bacillus laterosorus	Basiliskamides B (20)	 **20**	MIC = 3.1 μg/mL	Barsby *et al.*, 2002

Table 1 (cont.)

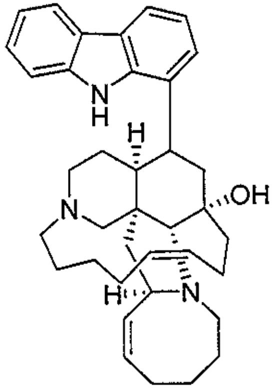
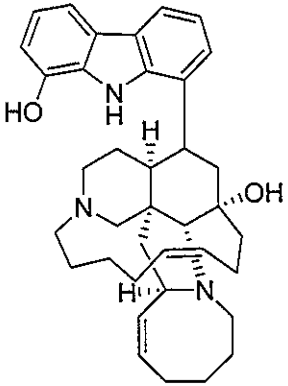
Sources	Names	Structures	Antitubercular activity	References
Haliclona sp.	Manzamine A (21)	 21	MIC = 1.53 $\mu g/mL$	Youssef *et al.*, 2002
Haliclona sp.	(+)-18-Hydroxy-manzamine A (22)	 22	MIC = 0.91 $\mu g/mL$	Youssef *et al.*, 2002

Table 1 (cont.)

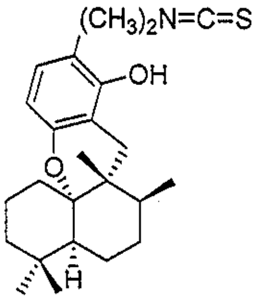
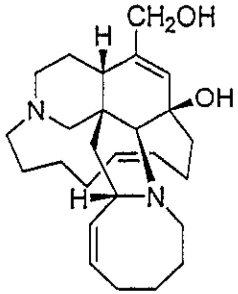
Sources	Names	Structures	Antitubercular activity	References
Smeno-spongia aurea	Aureol *N*,*N*-dimethyl-thiocarbamate (23)	 23	MIC = 6.25 μg/mL	Hu *et al.*, 2002
Haliclona sp.	Ircinol A (24)	 24	MIC = 1.93 μg/mL	Edrada *et al.*, 2002

Table 1 (cont.)

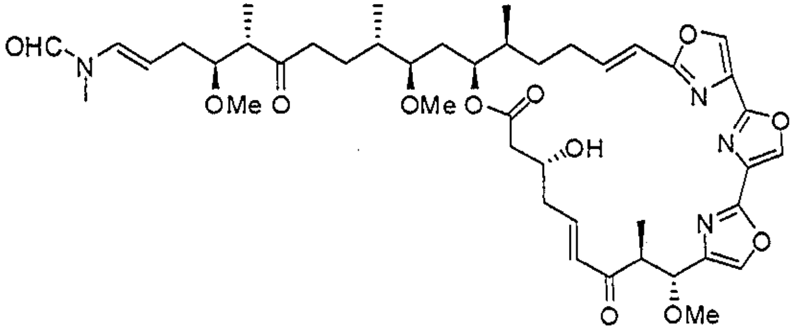
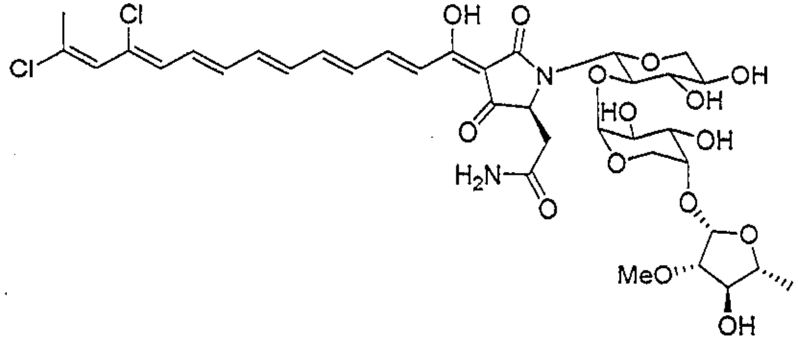
Sources	Names	Structures	Antitubercular activity	References
Halichondria sp.	Halichondramide (25)	 25	MIC = 0.2 μg/mL	Donia and Hamann, 2003
Siliquaria-spongia japonica	Aurantoside B (26)	 26	MIC = 0.16 μg/mL	Donia and Hamann, 2003

Table 1 (cont.)

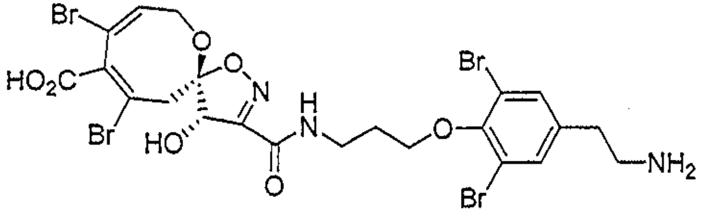
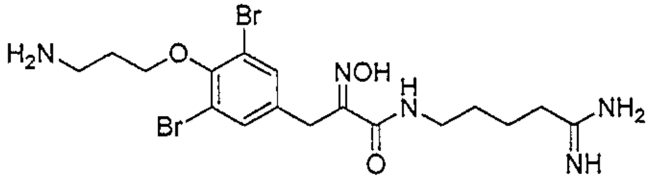
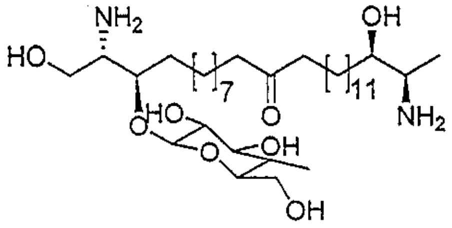
Sources	Names	Structures	Antitubercular activity	References
Pseudocera-tina sp.	Psammaplysin A (27)	 27	IC_{50} = 30.0 μM	Nicholas *et al.*, 2003
Oceanapia sp.	Oceanapia (28)	 28	IC_{50} = 3.0 μM	Nicholas *et al.*, 2003
Oceanapia sp.	Oceanapiside (29)	 29	IC_{50} = 10.0 μM	Nicholas *et al.*, 2003

Table 1 (cont.)

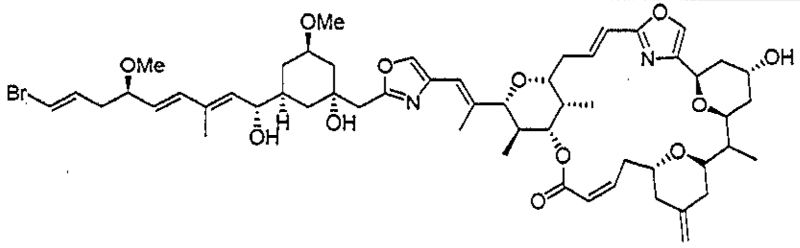
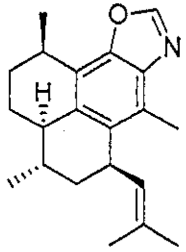
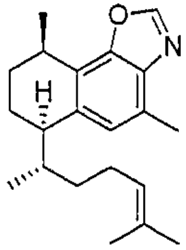
Sources	**Names**	**Structures**	**Antitubercular activity**	**References**
Phorbas sp.	Phorboxazole A (30)	 30	MIC = 0.1 μg/disk	Donia and Hamann, 2003
Pseudopterogorgia elisabethae	Pseudopteroxazole (31)	 31	MIC = 12.5 μg/mL	Rodriguez and Rodriguez, 2003
Pseudopterogorgia elisabethae	Seco-pseudopteroxazole (32)	 32	MIC = 12.5 μg/mL	Rodriguez and Rodriguez, 2003

Table 1 (cont.)

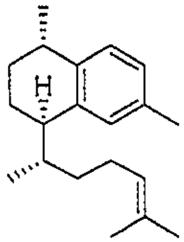
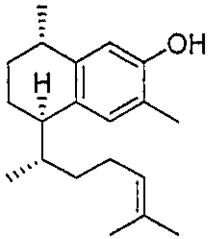
Sources	Names	Structures	Antitubercular activity	References
Pseudoptero-gorgia elisabethae	Erogorgiaene (33)	 33	MIC = 12.5 μg/mL	Rodriguez and Rodriguez, 2003
Pseudoptero-gorgia elisabethae	7-Hydroxy erogorgiaene (34)	 34	MIC = 6.25 μg/mL	Rodriguez and Rodriguez, 2003

Table 1 (cont.)

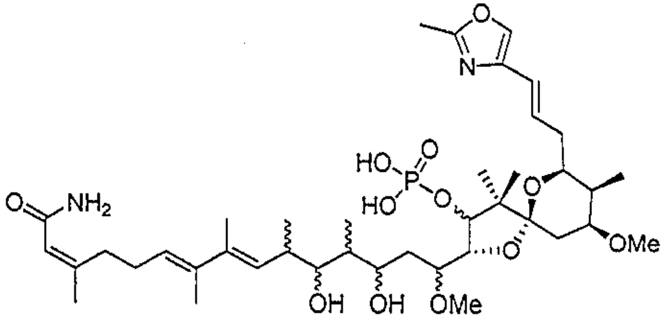
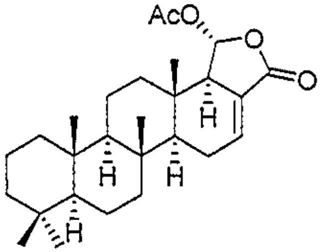
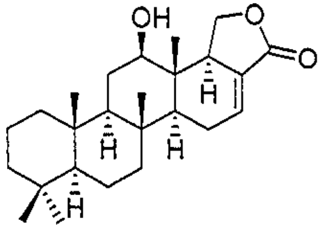
Sources	Names	Structures	Antitubercular activity	References
Theonella swinhoei	Swinhoeiamide A (35)	 35	MIC = 1 μg/mL	Rodriguez and Rodriguez, 2003
Brachiaster sp.	12-Deacetoxy scalarin 19-acetate (36)	 36	MIC = 4 μM	Wonganuchitmeta *et al.*, 2004
Brachiaster sp.	12-Deacetyl-12-*epi*-19-deoxyscalarin (37)	 37	MIC = 16 μM	Wonganuchitmeta *et al.*, 2004

Table 1 (cont.)

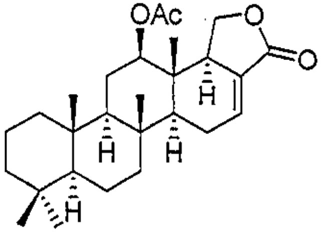
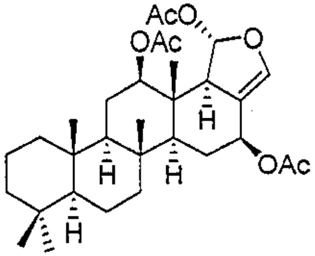
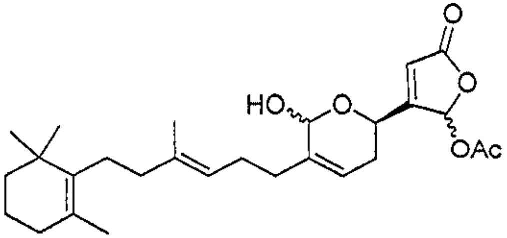
Sources	Names	Structures	Antitubercular activity	References
Brachiaster sp.	12-*epi*-19-Deoxyscalarin (38)	 **38**	MIC = 117 μM	Wonganuchitmeta *et al.*, 2004
Brachiaster sp.	Heteronemin acetate (39)	 **39**	MIC = 6 μM	Wonganuchitmeta *et al.*, 2004
Brachiaster sp.	Manoalide-25-acetate (40)	 **40**	MIC = 7 μM	Wonganuchitmeta *et al.*, 2004

Table **1** (cont.)

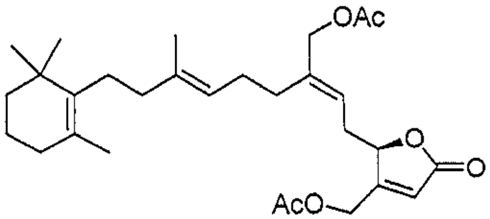
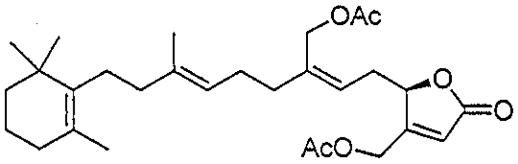
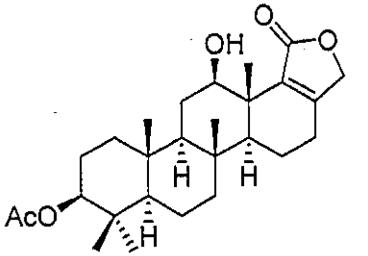
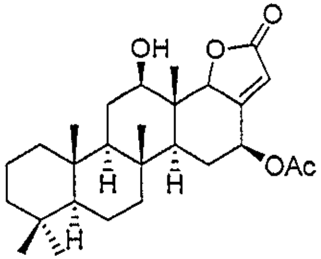
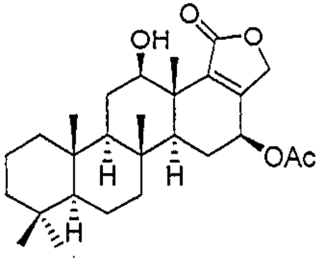
Sources	Names	Structures	Antitubercular activity	References
Brachiaster sp.	(*E*)- Neomanoalide 24,25-diacetates (41)	 **41**	MIC = 51 μM	Wonganuchitmeta *et al.*, 2004
Brachiaster sp.	(*Z*)-Neomanoalide 24,25-diacetates (42)	 42	MIC = 3.0 μM	Wonganuchitmeta *et al.*, 2004
Hyrtios sp.	3-Acetylsesterstatin 1 (43)	 43	MIC = 6.25 μg/mL	Youssef *et al.*, 2005

Table 1 (cont.)

Sources	Names	Structures	Antitubercular activity	References
Hyrtios sp.	16-*epi*-Scalarol butenolide (**44**)	 **44**	MIC = 6.25 μg/mL	Youssef *et al.*, 2005
Hyrtios sp.	Sesterstatin 7 (**45**)	 **45**	MIC = 6.25 μg/mL	Youssef *et al.*, 2005

1.2 The scalarin-type sesterterpenes

Sesterterpenoids are a group of pentaprenyl terpenoid substances derived directly from geranylfarnesyl diphosphate. Whereas the sources of sesterterpenes are widespread, having been isolated from various groups of organisms including terrestrial fungi, lichens, higher plants, and insects, the majority of sesterterpenes are of marine origins, particularly from sponges. Sesterterpenoids exhibit diverse biological properties, such as anti-inflammatory, cytotoxic, antifeedant, platelet-aggregation, and antimicrobial effects (Dewick, 1997).

Despite being relatively the smallest class of terpenoid compounds, the chemical structures of sesterterpenes are otherwise widely varied, ranging in term of numbers of carbocyclic systems from non-cyclic to as high as tetra-carbocyclic core skeletons. Among various groups of sesterterpenes, the scalarins, which are the focus of this investigation, is possibly the most common and most extensively studied class.

Chemically, scalarin-type sesterterpenes possess tetracarbocyclic core skeleton (Figure 2), occasionally with a furanoid extension attached to ring D. The compounds are exclusively marine, found primarily in the sponges, especially of the family Thorectidae. Certain scalarins were also reported from nudibranches, however with evident association to the sponges reportedly containing related scalarin precursors (Hanson, 1992). Please note that, due to the inconsistency among related publications, the atom numbering hereafter is referred to that shown in Figure 2 (Wonganuchitmeta *et al.*, 2004).

To date, there have been up to more than 100 naturally occurring scalarine-type sesterterpenes reported. These can be classified into two categories, namely furanoscalarins and non-furanoscalarins (Tables 2 and 3).

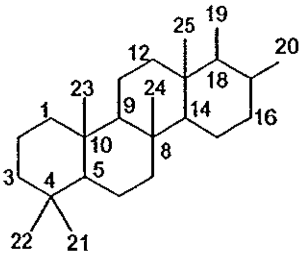


Figure 2 Scalarane skeleton

Table 2 Biological sources and activities of furanoscalarin-type sesterterpenoids

Compounds	Sources	Activities	References
Scalarin	*Cacospongia scalaris* (sponge)	N/A	Fattorusso *et al.*, 1972
Deoxoscalarin	*Spongia officinalis* (sponge)	N/A	Cimino *et al.*, 1973
Heteronemin	*Heteronema erecta* (sponge)	Cytotoxic (IC_{50} 1.2 μg/mL against KB); Antitubercular (MIC 6.25 μg/mL against strain $H_{37}Ra$)	Kazlauskas *et al.*, 1976; Doi *et al.*, 1993; El sayed *et al.*, 2000; Wonganuchitmeta *et al.*, 2004
12-*epi*-Scalarin	*Spongia nitens* (sponge)	N/A	Cimino *et al.*, 1977
Deoxoscalarin	*Spongia officinalis* (sponge)	N/A	Cimino *et al.*, 1977
12-*epi*-Deoxo scalarin	*Spongia nitens* (sponge)	N/A	Cimino *et al.*, 1977
Furoscalarol	*Cacospongia mollior* (sponge)	N/A	Cimino *et al.*, 1978
Scalardysin A	*Dysidea herbacea* (sponge)	N/A	Kashman *et al.*, 1979
Scalardysin B	*Dysidea herbacea* (sponge)	N/A	Kashman *et al.*, 1979

Table 2 (cont.)

Compounds	**Sources**	**Activities**	**References**
Scalarolide	*Spongia idea* (sponge)	N/A	Walker *et al.*, 1980
Scalarafuran	*Spongia idea* (sponge)	Cytotoxic (IC_{50} 7.2 μg/mL against KB)	Walker *et al.*, 1980; Doi *et al.*, 1993
23-Hydroxy-20-methylscalarolide	*Chromodoris sedna* (nudibranch)	N/A	Hochlowski and Faulkner, 1983
12-Deacetyl-20-methyl-12-*epi*-deoxoscalarin	*Chromodoris sedna* (nudibranch)	N/A	Hocchlowski and Faulkner, 1983
23-Hydroxy-20-methyldeoxoscalarin	*Chromodoris sedna* (nudibranch)	N/A	Hocchlowski and Faulkner, 1983
12-α-Acetoxy-19,20-epoxy-20-hydroxy-20,22-dimethyl scalarin	*Carteriospongia foliascens* (sponge)	Ichthyotoxic (LD_{50} 40 mg/mL against *Lebistes reticulatus*)	Braekman *et al.*, 1985
Heteronemin acetate	*Hyrtios erecta* (sponge)	Cytotoxic (IC_{50} 6.4 μg/mL against MCF-7); Antitubercular (MIC 6.25 μM against strain $H_{37}Ra$)	Crews and Bescansa, 1986; Wonganuchitmeta *et al.*, 2004
12-*epi*-Heteronemin acetate	*Hyrtios erecta* (sponge)	Cytotoxic (IC_{50} 2.7 μg/mL against KB)	Crews and Bescansa, 1986; Doi *et al.*, 1993

Table 2 (cont.)

Compounds	**Sources**	**Activities**	**References**
Deoxoscalarin acetate	*Spongia officinalis* (sponge)	N/A	De Giulio *et al.*, 1989
(-)-12-*epi*-Deoxoscalarin	*Spongia officinalis* (sponge)	N/A	De Giulio *et al.*, 1989
24-Acetoxy-12-deacetyl-12-*epi*-deoxoscalarin	*Hyatella intestinalis* (sponge)	N/A	Karoso *et al.*, 1989
16-Deacetyl-12-*epi*-scalafuran acetate	*Spongia officinalis* (sponge)	N/A	De Giulio *et al.*, 1989
Phyllofolactone A	*Phyllospongia foliascens* (sponge)	N/A	Zeng *et al.*, 1991
Phyllofolactone B	*Phyllospongia foliascens* (sponge)	N/A	Zeng *et al.*, 1991
Phyllofolactone B acetate	*Carteriospongia foliascens* (sponge)	N/A	Barron *et al.*, 1992
Phyllactone A	*Phyllospongia foliascens* (sponge)	Cytotoxic (IC_{50} 20 μg/mL against KB)	Fu *et al.*, 1992
Phyllactone B	*Phyllospongia foliascens* (sponge)	Cytotoxic (IC_{50} 20 μg/mL against KB)	Fu *et al.*, 1992
Phyllactone C	*Phyllospongia foliascens* (sponge)	N/A	Fu *et al.*, 1992

Table 2 (cont.)

Compounds	Sources	Activities	References
Phyllactone D	*Phyllospongia foliascens* (sponge)	N/A	Fu *et al.*, 1992
Phyllactone E	*Phyllospongia foliascens* (sponge)	N/A	Fu *et al.*, 1992
Phyllactone F	*Phyllospongia foliascens* (sponge)	N/A	Fu *et al.*, 1992
Phyllactone G	*Phyllospongia foliascens* (sponge)	N/A	Fu *et al.*, 1992
12-*O*-Deacetyl scalarin	*Hyrtios* sp. (sponge)	Nerve growth factor synthesis-stimulating (concentration 30-100 μg/mL)	Doi *et al.*, 1993
Isoscalarafuran A	*Spongia hispida* (sponge)	N/A	Davis and Capon, 1993
Isoscalarafuran B	*Spongia hispida* (sponge)	N/A	Davis and Capon, 1993
12-*O*-Deacetyl furoscalarol	*Hyrtios* sp. (sponge)	Nerve growth factor synthesis-stimulating (concentration 30-100 μg/mL)	Doi *et al.*, 1993
12-*epi*-Heteronemin	*Hyrtios erecta* (sponge)	N/A	Bourguet-Kondracki *et al.*, 1994

Table 2 (cont.)

Compounds	**Sources**	**Activities**	**References**
16-*O*-Deacetyl-16-*epi*-scalarol butenolide	*Hyrtios erecta* (sponge)	Cytotoxic (IC_{50} 0.4 μg/mL against P-388)	Ryu *et al.*, 1996
12-Deacetoxy-21-acetoxyscalarin	*Hyrtios erecta* (sponge)	Cytotoxic (IC_{50} 0.9 μg/mL against P-388)	Ryu *et al.*, 1996
12-*epi*-Acetylscalarolide	*Cacospongia scalaris* (sponge)	Cytotoxic (ED_{50} 1-2 μg/mL against P-388, Schabel, A-549, HT-29 and MEL-28)	Rueda *et al.*, 1997
19-Deoxyscalarin	*Cacospongia scalaris* (sponge)	N/A	Rueda *et al.*, 1997
16-Acetyl furoscalarol	*Cacospongia scalaris* (sponge)	Cytotoxic (ED_{50} 2.5-10 μg/mL against P-388, Schabel, A-549, HT-29 and MEL-28)	Rueda *et al.*, 1997
12-Deacetyl-12-*epi*-19-deoxy scalarin	*Hyrtios erecta* (sponge)	Cytotoxic (ED_{50} 2.9 μg/mL against P-388)	Pettit *et al.*, 1998
Sesterstatin 1	*Hyrtios erecta* (sponge)	Cytotoxic (IC_{50} 0.46 μg/mL against P-388)	Pettit *et al.*, 1998

Table 2 (cont.)

Compounds	Sources	Activities	References
Sesterstatin 2	*Hyrtios erecta* (sponge)	Cytotoxic (IC_{50} 4.2 μg/mL against P-388)	Pettit *et al.*, 1998
Sesterstatin 3	*Hyrtios erecta* (sponge)	Cytotoxic (IC_{50} 4.3 μg/mL against P-388)	Pettit *et al.*, 1998
12-*O*-Desacetyl furoscalar-16-one	*Cacospongia* sp. (sponge)	N/A	Cambie *et al.*, 1998
12-*O*-Acetyl-16-*O*-deacetyl-12,16-*epi*-scalarol butenolide	*Chromodoris inornata* (nudibranch)	Cytotoxic (IC_{50} 2.4 μg/mL against L1210)	Miyamoto *et al.*, 1999
Phyllofolactones C	*Phyllospongia foliascens* (sponge)	N/A	Fu *et al.*, 1999
Phyllofolactones D	*Phyllospongia foliascens* (sponge)	N/A	Fu *et al.*, 1999
12-*epi*-Deoxoscalarin-3-one	*Chromodoris inornata* (nudibranch)	Cytotoxic (IC_{50} 6.6 μg/mL against L1210)	Miyamoto *et al.*, 1999
Deoxoscalarin-3-one	*Chromodoris inornata* (nudibranch)	Cytotoxic (IC_{50} 0.95 μg/mL against L1210)	Miyamoto *et al.*, 1999

Table 2 (cont.)

Compound	**Source**	**Activities**	**References**
21-Acetoxydeoxoscalarin	*Chromodoris inornata* (nudibranch)	Cytotoxic (IC_{50} 0.35 μg/mL against L1210)	Miyamoto *et al.*, 1999
21-Hydroxydeoxoscalarin	*Chromodoris inornata* (nudibranch)	Cytotoxic (IC_{50} 4.1 μg/mL against L1210)	Miyamoto *et al.*, 1999
12-Deacetoxy-12-oxodeoxoscalarin	*Glossodoris atromarginata* (nudibranch)	Cytotoxic (25% of mortality against human thyroid carcinoma)	Fontana *et al.*, 1999
12-Dacetyl-12-*epi*-deoxoscalarin	*Glossodoris atromarginata* (nudibranch)	N/A	Fontana *et al.*, 1999
Hyrtiolide	*Hyrtios erecta* (sponge)	N/A	Miyamoto *et al.*, 2000
16-Hydroxy scalarolide	*Strepsichordaia aliena* (sponge)	N/A	Miyamoto *et al.*, 2000
Phyllofolactone H	*Strepsichordaia aliena* (sponge)	N/A	Jimenez *et al.*, 2000
Phyllofolactone I	*Strepsichordaia aliena* (sponge)	N/A	Jimenez *et al.*, 2000
Phyllofolactone J	*Strepsichordaia aliena* (sponge)	N/A	Jimenez *et al.*, 2000

Table 2 (cont.)

Compounds	Sources	Activities	References
Phyllofolactone K	*Strepsichordaia aliena* (sponge)	N/A	Jimenez *et al.*, 2000
12-Deacetyl-23-acetoxy-20-methyl-12-*epi*-deoxoscalarin	*Glossodoris sedna* (nudibranch)	N/A	Fontana *et al.*, 2000
3-Acetylsesterstatin 1	*Hyrtios erecta* (sponge)	N/A	Youssef *et al.*, 2002
19-Acetylsesterstatin 3	*Hyrtios erecta* (sponge)	N/A	Youssef *et al.*, 2002
Salmahyrtisol B	*Hyrtios erecta* (sponge)	N/A	Youssef *et al.*, 2002
12-*O*-Deacetyl scalarafuran	*Spongia* sp. (sponge)	Cytotoxic (IC_{50} 19.5 μg/mL against L1210)	Tsoukatou *et al.*, 2003
12-*O*-Deacetyl-12-*epi*-scalarin	*Spongia* sp. (sponge)	Cytotoxic (IC_{50} 2.3-15 μg/mL against L1210, HeLa, A549 and KB)	Tsoukatou *et al.*, 2003
12-*O*-Acetyl-16-*O*-methylhyrtiolide	*Spongia* sp. (sponge)	Cytotoxic (IC_{50} 2.2-15.6 μg/mL against L1210, HeLa, A549 and KB)	Tsoukatou *et al.*, 2003

Table 2 (cont.)

Compounds	Sources	Activities	References
16-Acetoxy-dihydrodeoxoscalarin	*Cacospongia scalaris* (sponge)	N/A	Tsoukatou *et al.*, 2003
12-α-*O*-Acetylhyrtiolide	*Hyrtios erectus* (sponge)	N/A	Qiu *et al.*, 2004
12-Deacetoxy scalarin-19-acetate	*Brachiaster* sp. (sponge)	Antitubercular (MIC 4 μM against strain $H_{37}Ra$)	Wonganuchitmeta *et al.*, 2004
Hyrtiosin A	*Hyrtios erecta* (sponge)	N/A	Yu *et al.*, 2005
Hyrtiosin B	*Hyrtios erecta* (sponge)	N/A	Yu *et al.*, 2005
Hyrtiosin C	*Hyrtios erecta* (sponge)	N/A	Yu *et al.*, 2005
Sesterstatin 6	*Hyrtios erecta* (sponge)	Cytotoxic (ED_{50} 0.17 μg/mL against P-388)	Pettit *et al.*, 2005
Sesterstatin 7	*Hyrtios erecta* (sponge)	Antitubercular (MIC 6.25 μg/mL against *M. tuberculosis* $H_{37}Rv$)	Youssef *et al.*, 2005
Hyatelone A	*Hyatella intestinalis* (sponge)	Cytotoxic (GI_{50} 5.4-9.2 μg/mL against MDA-MB-231 and HT29)	Hernandez-Guerrero *et al.*, 2006

Table 2 (cont.)

Compounds	Sources	Activities	References
Hyatelone B	*Hyatella intestinalis* (sponge)	N/A	Hernandez-Guerrero *et al.*, 2006
Hyatelone C	*Hyatella intestinalis* (sponge)	N/A	Hernandez-Guerrero *et al.*, 2006
Hyatolide A	*Hyatella intestinalis* (sponge)	N/A	Hernandez-Guerrero *et al.*, 2006
Hyatolide B	*Hyatella intestinalis* (sponge)	N/A	Hernandez-Guerrero *et al.*, 2006
Hyatolide E	*Hyatella intestinalis* (sponge)	N/A	Hernandez-Guerrero *et al.*, 2006
12α-Acetoxy-20,24-dimethyl-16,24-dioxoscalara-14,17-dien-24-ol-25,24-olide	*Phyllospongia papyracea* (sponge)	N/A	Li *et al.*, 2007

Table 2 (cont.)

Compounds	Sources	Activities	References
(12α,24*R*)-12-[(3-Hydroxypentanoyl)oxy] 20,24 -dimethyl-25-oxoscalara-15,17-dien-25,24-olide	*Phyllospongia papyracea* (sponge)	N/A	Lan *et al.*, 2007
(12α,24*S*)-12-[(3-Hydroxypentanoyl)oxy] 20,24 -dimethyl-25-oxoscalara-15,17-dien-25,24-olide	*Phyllospongia papyracea* (sponge)	N/A	Lan *et al.*, 2007

Note: N/A = Not available

Table 3 Biological sources and activities of non-furano scalarin-type sesterterpenoids

Compounds	Sources	Activities	References
Scalaradial	*Cacospongia mollior* (sponge)	Brine shrimp lethality (LD_{50} 0.18 μg/mL); Fish antifeedant (MIC 60 $\mu g/cm^2$ against *Carassius carassius*); PLA_2 inhibiting (IC_{50} 0.6 μM)	Cimino *et al.*, 1974; De Rosa *et al.*, 1994; Fontana *et al.*, 2000
Disidein	*Dissidea pellscens* (sponge)	N/A	Cimino *et al.*, 1975
12-*epi*-Scalaradial	*Spongia nitens* (sponge)	N/A	Cimino *et al.*, 1979
12,18-Di-*epi*-scalaradial	*Spongia nitens* (sponge)	N/A	Cimino *et al.*, 1979
Scalarherbacin A	*Dysidea herbacea* (sponge)	N/A	Kashman and Zviely, 1979
Scalarherbacin B	*Dysidea herbacea* (sponge)	N/A	Kashman and Zviely, 1979
Scalarherbacin A acetate	*Dysidea herbacea* (sponge)	N/A	Kashman and Zviely, 1979
Scalarherbacin B acetate	*Dysidea herbacea* (sponge)	N/A	Kashman and Zviely, 1979

Table 3 (cont.)

Compounds	Sources	Activities	References
24-Methyl-24,25-dioxoscalar-16-en-12β-yl-3-hydroxy butanoate	*Phyllospongia* spp. (sponge)	N/A	Kashman and Zviely, 1979
12α-Acetoxy-24-methyl-24-oxo-scalara -16-en-25-al	*Phyllospongia* spp. (sponge)	N/A	Kashman and Zviely, 1979
12-Deacetyl-12,18-di-*epi*-scalaradial	*Spongia idea* (sponge)	N/A	Walker *et al.*, 1980
12-Deacetyl scalaradial	*Cacospongia scalaris* (sponge)	Cytotoxic (ED_{50} 0.58 μg/mL against L-1210)	Yasuda and Tada, 1981
Foliaspongin	*Phyllospongin foliascens* (sponge)	Antiinflammatory (18.1% inhibition at concentration 10μg/disk utilizing chrio-allantoic membrane of chick embryo)	Kikuchi *et al.*, 1983
Sednolide	*Chromodoris sedna* (nudibranch)	N/A	Hocchlowski and Faulker, 1983
Sednolide-23-acetate	*Chromodoris sedna* (nudibranch)	N/A	Hocchlowski and Faulker, 1983

Table 3 (cont.)

Compounds	Sources	Activities	References
Hyrtial	*Hyrtios erecta* (sponge)	Antiinflammatory (43% decrease in ear weight of PMA induced inframmation at concentration 50 μg/mL)	Crews *et al.*, 1985; Crews and Bescansa, 1986
12-α-16-β-Diacetoxy-20,22-dimethyl-20-oxo-19-norscalarane	*Carteriospongia foliascens* (sponge)	Ichthyotoxic (LD_{50} 20 mg/L against *Lebistes reticulatus*)	Braekman *et al.*, 1985
12-α-Acetoxy-16-β-hydroxy-20,22-dimethyl-20-oxoscalar-19-al	*Carteriospongia foliascens* (sponge)	Ichthyotoxic (LD_{50} 5 mg/L against *Lebistes reticulatus*)	Braekman *et al.*, 1985
12-Deacetyl-12-*epi*-scalaradial	*Hyrtios erecta* (sponge)	N/A	Crews and Bescansa, 1986
12-Deacetyl-18-*epi*-12-oxoscala radial	*Chromodoris youngbluthi* (nudibranch)	N/A	Terem and Scheuer, 1986
Triacetyl disidein	*Disidea pallescens* (sponge)	N/A	Cimino *et al.*, 1987
6′-Chlorodisidein	*Disidea pallescens* (sponge)	N/A	Cimino *et al.*, 1987

Table 3 (cont.)

Compounds	Sources	Activities	References
6′-Brorodisidein	*Disidea pallescens* (sponge)	N/A	Cimino *et al.*, 1987
Phyllofoliaspongin	*Phyllospongia foliascens* (sponge)	Cytotoxic (84% inhibitory at 5 μg/mL against P-388); Antithrombocyte (IC_{50} 2.35 μg/mL against adenosine diphosphate)	Kitakawa *et al.*, 1989
Dehydrofoliaspongin	*Phyllospongia foliascens* (sponge)	N/A	Kitakawa *et al.*, 1989
Phyllofenone A	*Phyllospongia foliascens* (sponge)	N/A	Zeng *et al.*, 1991
Phyllofenone B	*Phyllospongia foliascens* (sponge)	Cytotoxic (IC_{50} 5 μg/mL against P-388)	Zeng *et al.*, 1991
12-Deacetoxy scalaradial	*Cacospongia mollior* (sponge)	Fish antifeedant (MIC 30 μg/cm^2 against Carassius carassius); Brine shrimp lethality (LD_{50} 0.77 μg/mL)	De Rosa *et al.*, 1994
18-*epi*-Scalaradial	*Cacospongia scalaris* (sponge)	Cytotoxic (ED_{50} 0.2-0.5 μg/mL against P-388, Schabel, A-549, HT-29 and MEL-28)	Rueda *et al.*, 1997

Table 3 (cont.)

Compounds	**Sources**	**Activities**	**References**
19-Dihydro scalaradial	*Cacospongia scalaris* (sponge)	Cytotoxic (ED_{50} 2-2.5 μg/mL against P-388, Schabel, A-549, HT-29 and MEL-28)	Rueda *et al.*, 1997
Norscalaral A	*Cacospongia scalaris* (sponge)	Cytotoxic (ED_{50} 1-2 μg/mL against P-388, Schabel, A-549, HT-29 and MEL-28)	Rueda *et al.*, 1997
Norscalaral B	*Cacospongia scalaris* (sponge)	Cytotoxic (ED_{50} 0.2-0.5 μg/mL against P-388, Schabel, A-549, HT-29 and MEL-28)	Rueda *et al.*, 1997
Norscalaral C	*Cacospongia scalaris* (sponge)	Cytotoxic (ED_{50} 2 μg/mL against P-388, Schabel, A-549, HT-29 and MEL-28)	Rueda *et al.*, 1997
25,26-Bishomo scalarin	*Cacospongia scalaris* (sponge)	N/A	De Rosa *et al.*, 1998
12-Deacetyl-Δ^{17}-hyral	*Hyrtios erectus* (sponge)	Antiproliferative (IC_{50} 2.82 μg/mL against KB)	Miyaoka *et al.*, 1998

Table 3 (cont.)

Compounds	**Sources**	**Activities**	**References**
Phyllofenone C	*Strepsichordaia aliena* (sponge)	N/A	Jimenez *et al.*, 2000
12-Deacetyl-23-acetoxy-20-methyl 12-*epi*-scalaradial	*Glossodoris sedna* (nudibranch)	Ichthyotoxic (0.1 ppm against *Gambusia affinnis*); Inhibited PLA_2 (IC_{50} 18 μM)	Fontana *et al.*, 2000
16-β-Acetoxy-20,24-dimethyl-12,24-dioxo-25-norscalarane	*Phyllospongia madagascarensis* (sponge)	N/A	Ponomaremko *et al.*, 2004
12-β-Hydroxy-20,24-dimethyl-13,18-oxa-25-norscalarane	*Phyllospongia madagascarensis* (sponge)	N/A	Ponomaremko *et al.*, 2004
Mooloolabenes A	*Hyattella intestinalis* (sponge)	Cytotoxic (IC_{50} 0.8 $\mu g/mL$ against P-388)	Somerville *et al.*, 2006
Mooloolabenes B	*Hyattella intestinalis* (sponge)	Cytotoxic (IC_{50} 1.2 $\mu g/mL$ against P-388)	Somerville *et al.*, 2006
Mooloolabenes C	*Hyattella intestinalis* (sponge)	N/A	Somerville *et al.*, 2006

Table 3 (cont.)

Compounds	**Sources**	**Activities**	**References**
12-α-Acetoxy-16-α-(3′-hydroxy pentanoyloxy)-24-oxoscalaran-25-β-oic acid	*Phyllospongia papyracea* (sponge)	N/A	Li *et al.*, 2007
12-α-Acetoxy-20,24-dimethyl-24-oxoscalar-16-en-25-β-oic acid	*Phyllospongia papyracea* (sponge)	N/A	Li *et al.*, 2007
25-Nor-12-α-acetoxy-20,24-dimethyl-24-oxoscalar-16-en-18-β-ol	*Phyllospongia papyracea* (sponge)	N/A	Li *et al.*, 2007
(12α,16β)-12-Acetoxy-16-hydroxy-20,24-dimethyl-25-nor scalar-17-en-24-one	*Phyllospongia papyracea* (sponge)	Cytotoxic (IC_{50} 5 μg/mL against P-388)	Lan and Li, 2007

Note; N/A = not available

1.3 Background and objectives

Thai marine bioresoures, considered to be among the most diverse and most abundant of the world, have long been recognized as one of the promising sources of new drugs and biologically active agents. The investigation towards new drug discovery from Thai marine natural products has been initiated, and various groups of biologically active compounds are now investigated. Among these include the antitubercular sesterterpenes of the scalarin family. Recently, one of the colleagues at Faculty of Pharmaceutical Sciences, Prince of Songkla University, isolated a series of antitubercular scalarins, of which heteronemin predominated as major components, from the sponge *Brachiaster* sp. In order to gain an indepth knowledge towards the further development of such compounds, various derivatives, obtained either from natural source or from chemical derivatization, are needed. The objectives of this investigation are therefore as the followings;

1) to isolate the minor sesterterpene derivatives, namely the scalarin-family, from the sponge *Hyrtios* sp.,

2) to modify the chemical structures of the scalarins, based on the main skeleton of heteronemin, especially toward the functionalities presumably affecting the biological activities, and

3) to determine the biological activities and the activity profiles of the derivatives obtained according to (2).

CHAPTER 2

EXPERIMENTAL

2.1 General

Unless stated otherwise, all chemicals are used as purchased. All solvents for general purposes and chromatography were commercial grade and were re-distilled prior to use. Thin layer chromatography (TLC) was performed on Merck® pre-coated silica gel 60 F_{254} plates (0.20 mm thickness). Chromatographic separations were performed over the columns of SiO_2 60 (Merck®; particle size 0.04-0.06 mm). Visualization was done with observation under UV light (254 nm), and with vanillin spraying reagents (dark spot on white background). Melting point was recorded on a Fisher-Johns melting point apparatus, and was uncorrected. Optical rotations were measured on a Jasco P-1020 polarimeter (Department of Chemistry, Faculty of Science, Prince of Songkla University). Ultraviolet spectra were recorded on a Specord S 100 (Analytikjena) and UV-160A spectrophotometer (Shimadzu). Principle bands (λ_{max}) were recorded as wavelengths (nm) with log ε in methanol solutions. Infrared spectra were recorded on an FTS FT-IR spectrophotometer, and major bands (ν) were recorded in wave number (cm^{-1}). ^1H and ^{13}C NMR spectra were recorded on an FT-NMR, Varian® Unity Inova 500 spectrometer (Germany). Chemical shifts are recorded in parts per million (δ) relative to the solvent signals of either C_6D_6 (7.15 ppm of residual C_6D_5H for ^1H NMR, and 128.0 ppm for ^{13}C NMR) or $CDCl_3$ (7.26 ppm of residual $CHCl_3$ for ^1H NMR, and 77.0 ppm for ^{13}C NMR). The EI-MS and ESI-TOF-MS were performed using a MAT 95 XL and Micromass LCT mass spectrometer, respectively.

For the chemical reactions, CH_2Cl_2 was distilled over calcium hydride to 4A molecular sieve, THF was distilled over sodium-benzoquinone to 4A molecular sieve, MeCN was distilled over calcium hydride to 4A molecular sieve, and pyridine was distilled over NaOH to NaOH pellets. All chemical reactions were conducted under dried nitrogen atmosphere in oven-dried, flame-dried vessels, using either oven-dried or seal-tight disposable glassware.

2.2 Animal material

The sponge *Hyrtios* sp. (Figure 3) was collected using SCUBA at the depth of 15-20 metres, from Koh-Tao, Surat-Thani Province, Thailand, (10° 07.569′ N; 99° 48.665′ E) in May, 2005. The sponge material was frozen immediately and kept frozen at −20 °C until extraction. The sponge was identified as *Hyrtios* sp. (class Demospongiae, order Dictyoceratida, family Thoractidae) by Dr. Somchai Bussarawit of the Phuket Marine Biology Center, Phuket, Thailand. The texture of the sponge is firmly incompressible with a dense, spongy interior. Some fine white fibres are apparent macroscopically. The surface is slightly glossy with fine conules close together. The colors were black-brown exterior and dark-brown interior. Primary and secondary fibres are cored with detritus. The primary fibres are fasciculate under the conules, up to 0.25 cm wide. Some secondary fibres, 0.05 cm wide, are not cored. The voucher specimen (PMBC 24608) was deposited at the Phuket Marine Biology Center, Phuket, Thailand.

Figure 3 The sponge *Hyrtios* sp.

2.3 Bioactivity determination

2.3.1 Antitubercular activity

The antitubercular activity was assessed against *Mycobacterium tuberculosis* $H_{37}Ra$ (non-virulent strain) using the microplate alamar blue assay (Collins and Franzblau, 1997) with the service provided by the Central Bioassay Lab, National Center for Genetic Engineering and Biotechnology (BIOTEC), Pathumthani. Initial sample dilutions were prepared in DMSO, and subsequent two-fold dilutions, starting from 200 μg/mL, were performed in 0.1 mL of 7H9GC in the microplate. BACTEC 12B-passaged inocula were initially diluted two fold in 7H9GC, and 0.1 mL of this inoculum was added to each well. Subsequent determination of bacterial titers yielded 2.5×10^6 CFU/mL in plate wells for $H_{37}Ra$. Frozen inocula were initially diluted 20 fold in BACTEC 12B medium followed by a 50 fold dilution in 7H9GC. Addition of 0.1 mL to wells resulted in final bacterial titer of 5×10^4 CFU/mL. Additional control wells consisted of bacteria only (B) and medium only (M). Plates were incubated at 37 °C. Starting on day 4 of incubation, 20 μL of 10% alamar blue solution and 12.5 μL of 20% Tween 80 were added to one B well and one M well, and plates were reincubated at 37 °C. Wells were observed at 12 and 24 hours for a color change from blue to pink. If the B wells became pink within 24 hours, alamar blue reagent was added to the entire plate. If the wells remained blue, additional M and B wells were tested daily until a color change occurred, at which time the reagents were added to all remaining wells. Plates were then incubated at 37 °C and resulted colors were recorded at 24 hours post-reagent addition. For the positive control, isoniazid, rifampicin, and kanamycin surfate were used as standard drugs. The MICs of agents in the test system were 0.02, 0.04, and 2.58 μM, respectively. Visual MICs were defined as the lowest concentration of samples that prevented a color change from blue to pink.

2.3.2 Cytotoxic activity

The cytotoxic determination was kindly supported by Assist. Prof. Dr. Supreeya Yuenyongsawad of Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University. The cell line utilized as the targeted cell line in this test was MCF-7 (breast adenocarcinoma). The sulphorhodamine B (SRB) assay was used in this study to assess growth inhibition. The following protocol is modified from that originally described by Skehan *et al* (1990).

For the assay, monolayered culture in a 96-well microtiter plate (2×10^3 cells/well) was treated with a serial dilution (at least five concentrations) of each sample in suitable culture medium. All the plates were incubated according to the reported condition for seven days, at the midway of which time the medium was refreshed once (exposure time 72 hours). On the seventh day of culture period, ice-cold 40% trichloroacetic acid (TCA) was added to each well. The plates were washed five times with water. The TCA-fixed cells were stained for 30 min with 0.4% SRB in 1% acetic acid. The plates were washed five times with 1% acetic acid and allowed to dry overnight. Once dried, bound dye was dissolved with 10 mM Tris base for 20 min on a gyratory shaker. Survival percentage was measured via the intensity of the resulted purplish-pink color at 492 nm (Power Wave X plate reader). The IC_{50} values were calculated from the dose-response curves and referred to that of standard camptothecin (Aldrich, IC_{50} toward MCF-7 0.72 nM).

2.4 Isolation and purification

The sponge *Hyrtios* sp. was collected in April 2005. The freeze-dried sponge (101 g) was crushed and macerated exhaustively (3 L × 5) in methanol to yield a crude extract after evaporation of the solvent in vaccuo. The crude extract was partitioned with the procedure modified from Kupchan and Tsou (1973), and hexane-, CH_2Cl_2- and *n*-BuOH-soluble materials were obtained (2.60 g, 2.57%; 4.29 g, 2.25%; 6.01 g, 5.95%, respectively). The hexane- and CH_2Cl_2-fractions, which exhibited antitubercular activity against *Mycobacterium tuberculosis* $H_{37}Ra$, both with MIC of 25 μg/mL, were chosen for the isolation of the bioactive compounds.

The hexane-fraction (2.60 g) was separated using the quick chromatographic technique over a column of SiO_2 (hexane:EtOAc:acetone 7:2:1). Fractions with similar chromatographic pattern were combined to yield three main fractions. The first fraction was recrystallized from hexane:EtOAc mixture (5:1) to afford compound **1** (317 mg, 12.2% of hexane fraction), which was identified to be heteronemin. The second fraction (48 mg) was subjected to the silica gel column chromatography (1.2 × 40 cm) and eluted with hexane-EtOAc gradient from (7:3 to 5:5) to yield compound **46** (5 mg; 0.2% of hexane fraction).

The CH_2Cl_2 fraction (4.29 g) was fractionated using the quick chromatographic technique over a column of SiO_2 (hexane:EtOAc:acetone 7:2:1). Fractions containing compound **1** as major component were all combined, and were subjected to a recrystallization process to yield **1** (1.08 g; 20.3% of CH_2Cl_2 fraction).

2.5 Chemical characteristics

Heteronemin (1). White needles, mp: 180-181 °C; $[\alpha]_D$: -39.1° (c = 0.11, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 216 (3.65) nm; IR (neat) ν_{max} 3490, 1742, 1236 cm^{-1}; HREIMS: *m/z* $[M]^+$ 428.2938 (calcd for $C_{27}H_{40}O_4$, 428.2927); ^1H and ^{13}C NMR (C_6D_6, 500 MHz for ^1H), see Table 5.

12-*epi*-Deacetyl-19*α*-acetoxy-20*α*-methoxyscalaran (46). White solid; $[\alpha]_D$: +7.1° (c = 0.21, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 208 (4.47), 218 (4.45) nm; IR (neat) ν_{max} 3444, 1734 cm^{-1}; HRESIMS: *m/z* $[M+Na]^+$ 483.3071 (calcd for $C_{28}H_{44}O_5Na$, 483.3087); ^1H and ^{13}C NMR ($CDCl_3$, 500 MHz for ^1H), see Table 4.

Heteronemin acetate (39). Acetylation of **1** (30 mg; 0.061 mmol) was accomplished using standard acetylation condition; i.e., stirring with Ac_2O (2 mL; 21.2 mmol) in pyridine (1 mL) at room temperature for 18 h. The mixture was purified over a SiO_2 column (EtOAc:hexane 3:7) to yield **39** (26 mg; 79%). White solid; $[\alpha]_D$: -155.9° (c = 0.32, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 212 (4.48) nm; IR (neat) ν_{max} 1741, 1237, 1023 cm^{-1}; HRESIMS: *m/z* $[M+Na]^+$ 553.3121 (calcd for $C_{31}H_{46}O_7Na$, 553.3142); ^1H and ^{13}C NMR (C_6D_6, 500 MHz for ^1H), see Table 11.

12-Deacetyl-12-*epi*-scalaradial (47). To the ice-cold solution of **1** (350 mg; 0.717 mmol) in MeCN (15 mL) was added $BF_3 \cdot OEt_2$ (180.97 μL; 1.434 mmol) and a few drops of water. The solution was stirred at 0 °C for 1 h, at which time $NaHCO_3$ solution was added. The mixture was extracted with EtOAc (50 mL × 3) and purified over a SiO_2 column (acetone:EtOAc:hexane 1:2:7) to yield **47** (267 mg; 96%), along with **48** as minor component (4 mg; 3%). White solid; $[\alpha]_D$: +15.5° (c = 0.13, $CHCl_3$); UV

(MeOH) λ_{max} (log ε) 225 (4.56) nm; IR (neat) ν_{max} 1745, 1704, 1233 cm^{-1}; HREIMS: *m/z* [M]$^+$ 386.2818 (calcd for $C_{25}H_{38}O_3$, 386.2821); ^{1}H and ^{13}C NMR (C_6D_6, 500 MHz for ^{1}H), see Table 6.

12-Deacetyl-12,18-di-*epi*-scalaradial (48). White solid; $[\alpha]_D$: +172.6° (*c* = 0.15, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 226 (3.86) nm; IR (neat) ν_{max} 1732, 1718, 1264 cm^{-1}; HREIMS: *m/z* [M]$^+$ 386.2803 (calcd for $C_{25}H_{38}O_3$, 386.2821); ^{1}H and ^{13}C NMR (C_6D_6, 500 MHz for ^{1}H), see Table 7.

12-Deacetyl-12-*epi*-deoxoscalarin (49). To a solution of **47** (38 mg; 0.0984 mmol) in THF (10 mL) was added LAH (2.0 M in THF, 0.6 mL; 0.590 mmol). The mixture was refluxed for 1 h. The solution was cooled in ice, and the reaction mixture was quenched with EtOAc (20 mL), which was added dropwise with vigorous stirring, followed by the addition of 10 mL of H_2O. The mixture was purified over a SiO_2 column (EtOAc:hexane 3:7) to yield **49** (4 mg; 11%). White solid; $[\alpha]_D$: +77.0° (*c* = 0.17, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 207 (3.88), 224 (3.69), 273 (3.24) nm; IR (neat) ν_{max} 3400, 1453, 1394 cm^{-1}; HRESIMS: *m/z* [M+Na]$^+$ 411.2866 (calcd for $C_{25}H_{40}O_3Na$, 411.2875); ^{1}H and ^{13}C NMR (C_6D_6, 500 MHz for ^{1}H), see Table 8.

12-Oxoheteronemin (50). To the suspended PCC/SiO_2 (1:1; 43 mg, 0.204 mmol of PCC) in CH_2Cl_2 (5 mL) was added **1** (50 mg, 0.102 mmol). The reaction mixture was stirred at room temperature for 2.5 h, and the resulting brown suspension was filtered by mean of celite, then washed with Et_2O (50 mL). The purification was done by means of SiO_2 column chromatography (acetone:EtoAc:hexane 1:2:7) to yield **50** (49 mg; 98%). White solid; $[\alpha]_D$: +2.4° (*c* = 0.15, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 220 (4.41) nm; IR (neat) ν_{max} 3485, 1736, 1242, 752 cm^{-1}; HREIMS: *m/z* [M$-C_2H_4O_2$]$^+$ 426.2788 (calcd for $C_{27}H_{38}O_4$, 426.2770); ^{1}H and ^{13}C NMR (C_6D_6, 500 MHz for ^{1}H), see Table 9.

12-Deacetyl-12-oxoscalaradial (51). The oxidation of **47** (30 mg; 0.130 mmol) was accomplished using the similar protocol as described for **50**. The purification was done over a SiO_2 column (acetone:EtOAc:hexane 1:2:7) to yield **51** (26 mg; 87%). White solid; $[\alpha]_D$: +90.2° (*c* = 0.12, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 223 (5.02) nm; IR (neat) ν_{max} 1702 cm^{-1}; HREIMS: *m/z* [M]$^+$ 384.2668 (calcd for $C_{25}H_{36}O_3$, 384.2664); ^{1}H and ^{13}C NMR (C_6D_6, 500 MHz for ^{1}H), see Table 10.

12-*epi*-Scalaradial (52). Acetylation of **47** (23 mg; 0.0596 mmol) was accomplished using standard acetylation condition, i.e., using Ac_2O (2 mL) in pyridine (1 mL) at room temperature for 18 h. The mixture was dried under reduced pressure. After the usual workup, the reaction mixture was purified over a SiO_2 column (EtOAc:hexane 3:17) to yield **52** (8 mg; 31%). White solid; $[\alpha]_D$: +46.5° (*c* = 0.28, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 210 (3.14), 223 (3.18) nm; IR (neat) ν_{max} 1735, 1241 cm^{-1}; HRESIMS: *m/z* [M+Na]$^+$ 451.2837 (calcd for $C_{27}H_{40}O_4Na$, 451.2877); ^{1}H and ^{13}C NMR (C_6D_6, 500 MHz for ^{1}H), see Table 12.

Pyrolysis of 1. Dry portion of **1** (30 mg, 0.061 mmol) was placed under a dry nitrogen atmosphere and exposed to the 220 °C temperature for 2.5 min. The vessel was then allowed to cool to room temperature, and the mixture was purified over a SiO_2 column (EtOAc:hexane 1:4) to yield compounds **53**, **54** and **48** (4 mg, 16%, 2 mg, 8% and 4, 17%, respectively).

Scalarafuran (53). White solid; $[\alpha]_D$: +8.6° (*c* = 0.21, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 206 (3.50), 278 (2.90) nm; IR (neat) ν_{max} 1457, 1265 cm^{-1}; HREIMS: *m/z* [M]$^+$ 428.2965 (calcd for $C_{27}H_{40}O_4$, 428.2986); ^{1}H and ^{13}C NMR (C_6D_6, 500 MHz for ^{1}H), see Table 13.

16-Deacetoxy-15,16-dehydroscalarafuran (54). White solid; $[\alpha]_D$: +15.3° (*c* = 0.21, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 208 (3.41), 218 (3.44), 273 (2.46) nm; IR (neat) ν_{max} 3443, 1735, 1046 cm^{-1}; HREIMS: *m/z* [M]$^+$ 368.2710 (calcd for $C_{25}H_{36}O_2$, 368.2715); ^{1}H and ^{13}C NMR (C_6D_6, 500 MHz for ^{1}H), see Table 14.

CHAPTER 3

RESULTS AND DISCUSSION

The investigation reported in this thesis is aiming toward the establishment of chemical library for the marine-derived sesterterpenes, namely the scalarin derivatives. The activity profiles that are the focal points of this report included the antitubercular activity and cytotoxicity. The sources of the targeted compounds are both the natural products from the sponge *Hyrtios* sp., and the derivatized products using heteronemin as starting material. The discussion in this chapter, therefore, will be presented in three main parts; the isolation and structure determination of the natural products, the derivatization of the sesterterpenes, and the structure-activity relationship of the scalarin derivatives.

3.1 Isolation and structure elucidation

3.1.1 Isolation

The Thai sponge, *Hyrtios* sp. was collected at the depth of 15-20 metres, from Koh-Tao, Suratthani Province, Thailand, in May, 2005. The specimen was extracted with MeOH exhaustively (3 L × 5), then partitioned with organic solvents to yield hexane-, CH_2Cl_2- and *n*-BuOH-soluble materials (2.6, 4.3, and 6.0 g, respectively). The hexane extract was fractionated, and the major active compound, heteronemin (**1**), was obtained (317 mg), along with a new compound, 12-*epi*-deacetyl-19α-acetoxy-20α-methoxyscalaran (**46**) (5 mg). The CH_2Cl_2 fraction was subjected to the chromatographic isolation to yield heteronemin (**1**) as the major component (1.08 g).

3.1.2 Structure elucidation

3.1.2.1 12-*epi*-Deacetyl-19α-acetoxy-20α-methoxyscalaran (46)

Compound 46 was obtained as white amorphous solid (5 mg) from the hexane-soluble material using the chromatographic technique over a SiO_2 column (hexane-EtOAc gradient from 7:3 to 5:5).

Compound 46 has a molecular formula of $C_{28}H_{44}O_5$ as established by means of the ESI mass spectrum, which shows a molecular ion peak at *m/z* 483 ($[M+Na]^+$). This was confirmed by the high resolution ESI mass spectrum, which also showed a peak of $[M+Na]^+$ at 483.3071 (calcd for $C_{28}H_{44}O_5Na$ 483.3087). The proposed molecular formula requires the unsaturation degree of 7, one of which is a carbonyl, and another is an olefin; therefore five rings are needed. The infrared absorptions at ν 3444 and 1734 cm^{-1} are consistent with the presence of the hydroxyl and ester carbonyl functionalities. The UV spectrum shows the maximal absorption at λ 208 nm (log ε 4.47).

The ^{1}H NMR spectrum of 46 (Table 4, Figure 4) exhibited five aliphatic methyl singlets (δ 0.69, H-25; 0.74, H-22; 0.78, H-21; 0.78, H-23 and 0.83, H-24), one methoxy group (δ 3.32, s, 3H; H-28), one acetate methyl (δ 2.04, s, 3H; H-27), one olefinic proton (δ 5.79; H-16) and two acetal protons (δ 5.09; H-20, and 6.45; H-19, respectively), along with multiplet methylene signals, integrated to belong to 14 protons. The ^{13}C NMR spectrum (Table 4, Figure 5) revealed 28 carbons, including one methoxy group (δ 54.6), one ester carbonyl (δ 171.0), six quarternary carbons, eight methines, seven methylenes, and six methyls, accounted for 43 hydrogen atoms; one exchangeable proton as a hydroxyl group, therefore, complemented the proposed molecular formula.

Interpretation of ^{1}H-^{1}H COSY cross peaks in the aliphatic methylene region led to four partial structures, including fragment A [δ 0.81 (m, H-1ax), 1.56 (m, H-1eq), 1.29 (m, H-2ax), 1.45 (m, H-2eq), 1.07 (m, H-3ax) and 1.24 (m, H-3eq)]; fragment **B** [δ 0.72 (dd, J = 14.0, 2.0 Hz, H-5), 1.26 (m, H-6ax), 1.40 (m, H-6eq), 0.85 (m, H-7ax) and 1.51 (m, H-7eq)]; fragment C [δ 0.86 (m, H-9), 1.33 (m, H-11ax), 1.64 (m, H-11eq) and 3.38 (dd; J = 12.0, 4.5 Hz, H-12)]; and fragment **D** [δ

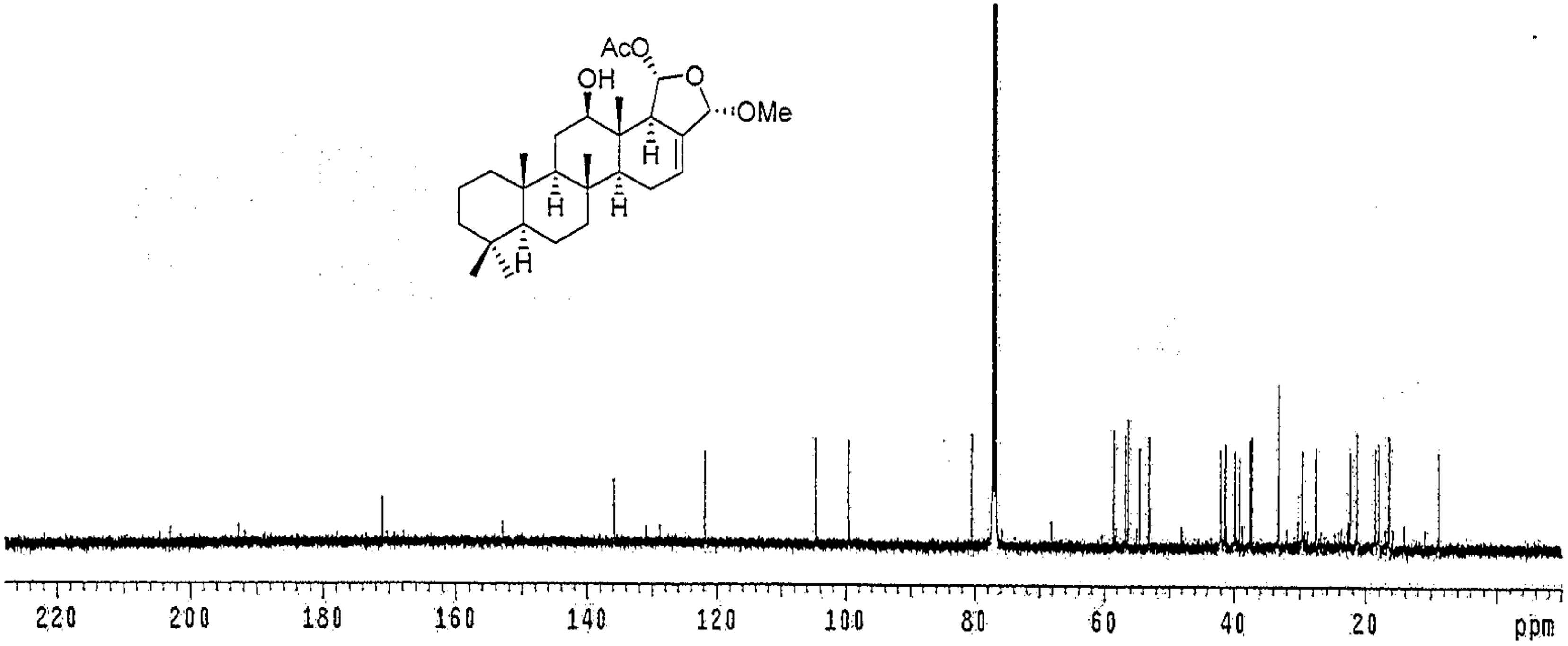


Figure 4 ^{13}C NMR spectrum of compound **46** ($CDCl_3$; 125 MHz)

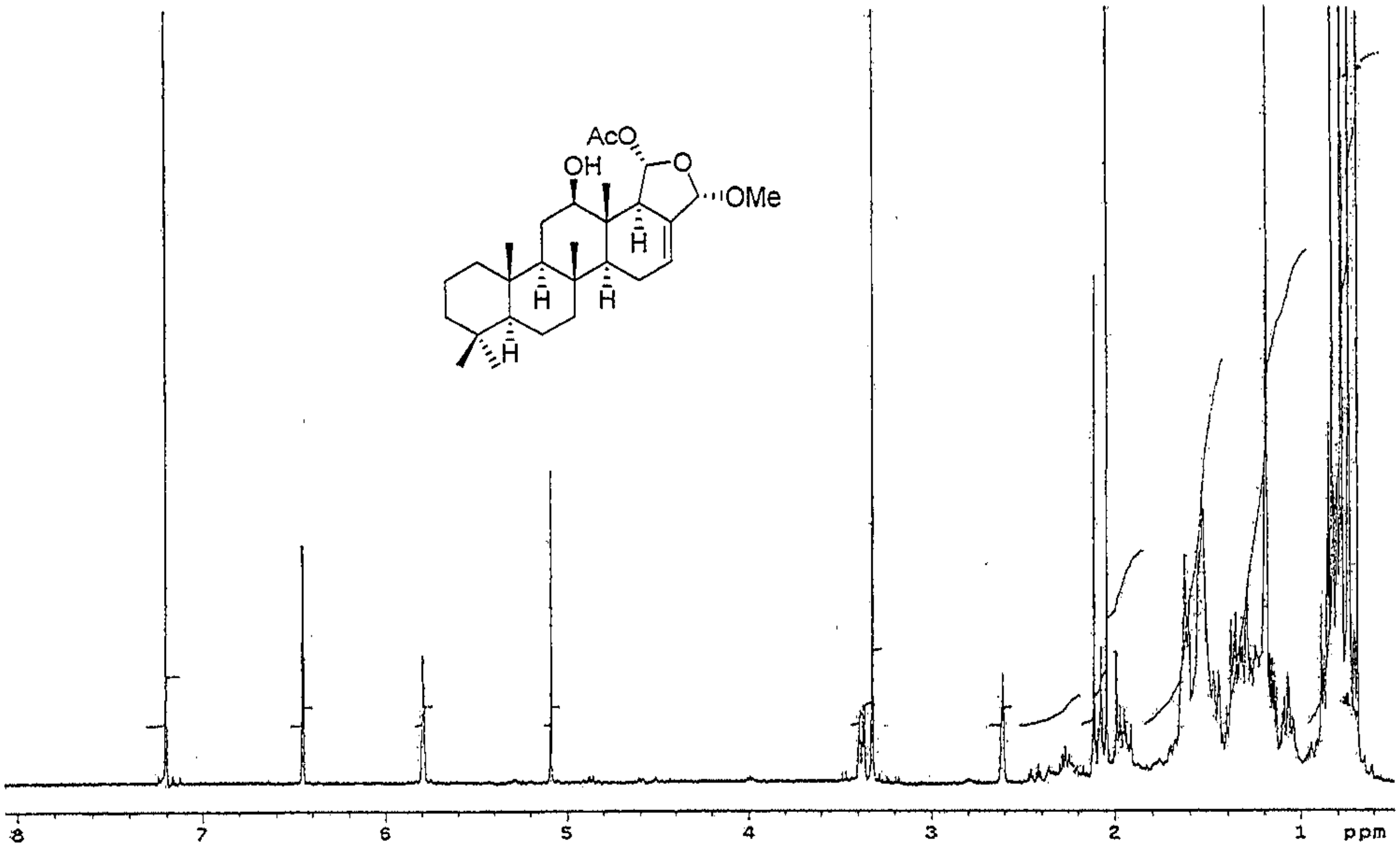
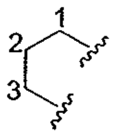


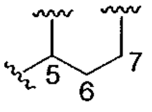
Figure 5 ^{1}H NMR spectrum of compound 46 ($CDCl_3$; 500 MHz)

1.15 (dd; J = 11.0, 5.5 Hz, H-14), 1.95 (m, H-15ax), 2.08 (m, H-15eq) and 5.79 (dd; J = 6.5, 3.0 Hz, H-16)] as shown below. The assignments of axial and equatorial orientations as stated were carried out by means of the coupling constant analysis, along with the observation for the chemical shift of each signal. The axial proton, shielded by the 1,3-diaxial repulsion, is generally found at a comparatively higher-fielded chemical shift than that of its equatorial counterpart (Crews and Bescanza, 1986).

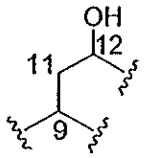
The remaining resonances, which include those of an acetal proton at δ 6.45 (d; J = 3.0 Hz, H-19) coupled to a methine signal at δ 2.61 (t; J = 3.0 Hz, H-18), a methine proton at δ 5.09 (s, H-20), and disubstituted olefinic carbon at δ 135.8 (C-17), belonged to the γ-oxygenated lactol. An acetoxy group (δ_C 171.0, C-26; δ_H 2.04, s, 3H; H-27) was placed at C-19 based on the HMBC data. This structure part is shown as fragment E.



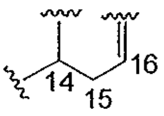
A



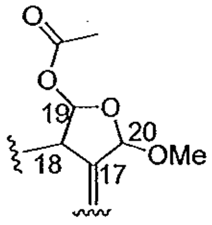
B



C



D



E

The crucial HMBC correlations (Figure 6) included those from C-3, C-4, and C-5 to H-21 and H-22; from C-1, C-9, and C-10 to H-23; from C-8, C-9, and C-14 to H-24; from C-13 and C-14 to H-25. Thus, the tetracarbocyclic moiety (rings A-D) was linked together. The HMBC correlations from C-20 to H-16 and from C-18 to H-14, H-16, and H-25 indicated that fragment E fused to ring D at C-17 and C-18. The structure of **46** was therefore proposed as 12-*epi*-deacetyl-19α-acetoxy-20α-methoxyscalaran, a new member of scalarane sesterterpenes, as shown.

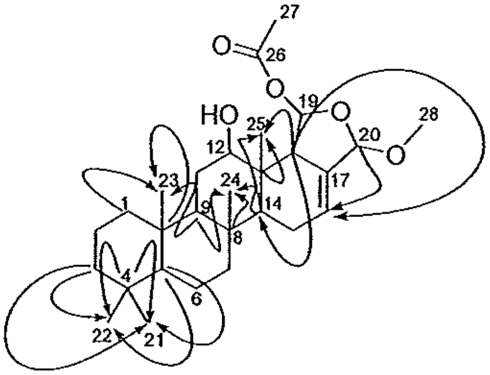


Figure 6 Chemical structure of **46** with crucial HMBC correlation (C → H).

The conformation of tetracarbocyclic ring system of **46** was determined via a close observation of the ^{13}C chemical shifts of ring junction methyls, i.e., C-23 (δ 16.5), C-24 (δ 17.3) and C-25 (δ 8.8), respectively. On the basis of the observation of ^{13}C shifts in the models by Crews and Bescansa (1986), ring junction methyls on *trans*-fused ring in a chair conformation are shielded (thus resonating at as low frequency as 20 ppm or lower) relative to those on *trans*-fused ring in a boat conformation and *cis*-fused ring of both conformation. Thus, the conformation of rings A, B and C was assigned as all *trans*. Consequently, H-5, H-9 and H-14 are all assigned as α and axial protons.

The configuration of C-18 was assigned on the basis of coupling constant analysis and were confirmed by the nOe observation (Figure 7). Allylic coupling between H-18 and H-16 (J = 3.0 Hz) suggested an axial orientation at H-18 (Pretsch *et al.*, 1989). Nuclear Overhauser enhancements among H-12, H-14 and H-18 indicated that all the three protons resided on the same α plane, all also in the axial orientation. A small enhancement at H-20 upon irradiation of H-19 also indicated that the two protons resided on the same plane. In order to propose the possible configuration of either protons, MM2 calculations of two possible conformers (Figure 8) were carried out. The major difference affecting the NMR spectrum of **46** was observed at H-20. Whereas conformer A, in which H-20 was at the α orientation, provided an approximately 154° allylic angle, that of conformer **B**, with the 20β orientation was rather close to 72°. Based on the absent allylic coupling between H-16 and H-20, which suggested the allylic angle close to 90°, the orientations of H-20 and H-19 were therefore proposed to be β as seen in Figure 8b. The absolute configuration as shown here was presumed to be coherent to that of heteronemin (see 3.1.2.2).

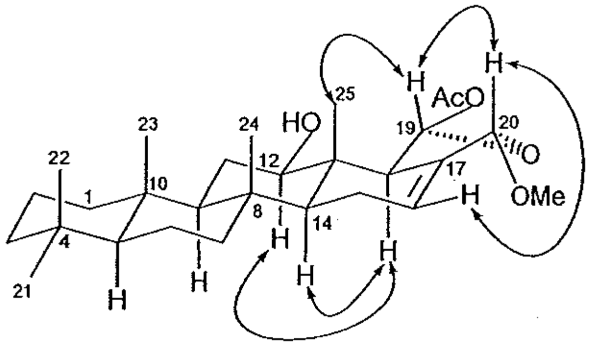
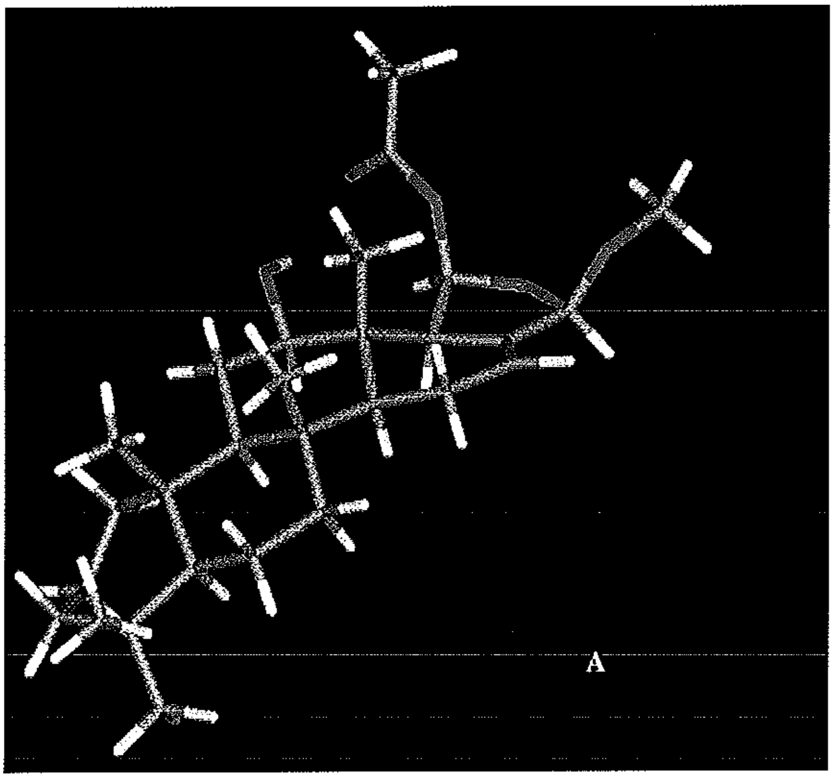
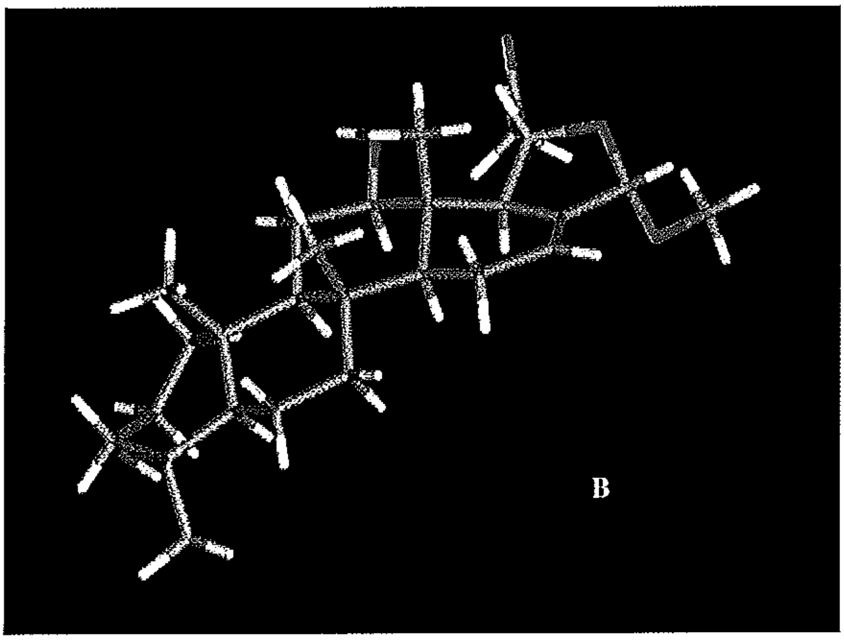


Figure 7 Selected nOe correlations of compound **46**



a



b

Figure 8 MM2-calculated conformers of **46**; (a) with 19α, 20α- and (b) with 19β, 20β- orientations

Table 4 ^{1}H and ^{13}C NMR spectral data and HMBC correlation of compound **46** ($CDCl_3$; 500 MHz for ^{1}H)

positions	^{1}H (mult.; *J* in Hz)	^{13}C (mult.)	HMBC correlation (C → H)
1 eq	1.56 (m)	39.9 (CH_2)	H-5, H-23
ax	0.81 (m)		
2 eq	1.45 (m)	18.0 (CH_2)	H-1
ax	1.29 (m)		
3 eq	1.24 (m)	41.3 (CH_2)	H-1, H-21, H-22
ax	1.07 (dt; 3.5, 13.0)		
4	-	33.3 (C)	H-21, H-22
5	0.72 (dd; 14.0, 2.0)	56.4 (CH)	H-1, H-7, H-21, H-22, H-23
6 eq	1.40 (m)	18.5 (CH_2)	H-5
ax	1.26 (m)		
7 eq	1.51 (m)	42.0 (CH_2)	H-9, H-24
ax	0.85 (m)		
8	-	37.3 (C)	H-6, H-7, H-9, H-11, H-14, H-24
9	0.86 (m)	58.6 (CH)	H-5, H-11, H-23, H-24
10	-	37.5 (C)	H-1, H-2, H-6, H-9, H-11, H-23
11 eq	1.64 (m)	27.6 (CH_2)	H-9
ax	1.33 (m)		
12	3.38 (dd; 12.0, 4.5)	80.5 (CH)	H-9, H-25
13	-	39.2 (C)	H-11, H-14, H-25
14	1.15 (dd; 11.0, 5.5)	53.1 (CH)	H-9, H-24, H-25
15 eq	2.08 (m)	22.4 (CH_2)	H-14
ax	1.95 (m)		
16	5.79 (dd; 6.5, 3.0)	121.8 (CH)	H-20
17	-	135.8 (C)	H-18, H-20
18	2.61 (t; 3.0)	56.8 (CH)	H-14, H-20, H-25
19	6.45 (d; 3.0)	99.6 (CH)	H-20, H-27

Table 4 (cont.)

positions	^{1}H (mult.; *J* in Hz)	^{13}C (mult.)	HMBC correlation (C → H)
20	5.09 (s)	104.6 (CH)	H-19, H-28
21	0.78 (s; 3H)	33.2 (CH_3)	H-3, H-5, H-22
22	0.74 (s; 3H)	21.3 (CH_3)	H-3, H-5, H-21
23	0.78 (s; 3H)	16.5 (CH_3)	H-9
24	0.83 (s; 3H)	17.3 (CH_3)	H-7, H-9, H-14
25	0.69 (s; 3H)	8.8 (CH_3)	H-14
26	-	171.0 (C)	H-19, H-27
27	2.04 (s; 3H)	21.3 (CH_3)	-
28	3.32 (s; 3H)	54.6 (CH_3)	H-20

3.1.2.2 Heteronemin (1)

Compound **1** was cystallized as white needles from the mixture of hexane:EtOAc (5:1). The molecular formula of 1 was proposed to be $C_{29}H_{44}O_6$ as observable in the EI mass spectrum, which showed a molecular peak at *m/z* 428 $[M]^+$, along with 44 protons and 29 carbons seen in the NMR spectra (Table 5). The proposed molecular formula was confirmed by the molecular peak at *m/z* 428.2938 in the HREI mass spectrum (calcd for $C_{29}H_{44}O_6$ 428.2927). Accordingly, the resulting degree of unsaturation of 8 was determined as two carbonyls, an olefin, and five ring systems. The major absorption band in the IR spectrum at 3490 cm^{-1} was assigned to the hydroxyl group, whereas those at ν 1742 and 1236 cm^{-1} were assigned to the ester functionality. The UV spectrum shows the absorption maximum at λ 216 nm (log ε 3.65).

The ^{13}C NMR spectrum (Figure 10) displayed 29 carbons including those of two ester carbonyls at δ 170.6 (C-26) and 169.4 (C-28), and a trisubstituted olefin composed of two carbons at δ 135.9 (C-20) and 101.8 (C-19). The ^{1}H NMR spectrum of 1 (Figure 11) displayed five tertiary methyl groups at δ 0.93 (H-25), 0.89 (H-21), 0.79 (H-22), 0.67 (H-23), and 0.60 (H-24), and two methyls of acetyl groups at δ 1.69 (H-29) and 1.67 (H-27).

The ^{1}H,^{1}H COSY spectrum of 1 indicated that the fragments similar to A, B and D of compound 46 were to be constructed. Fragment C, i.e., C-14 - C-16, differed from that of 46 in that a carbonol carbon (δ 69.6, C-16) replaced the olefinic

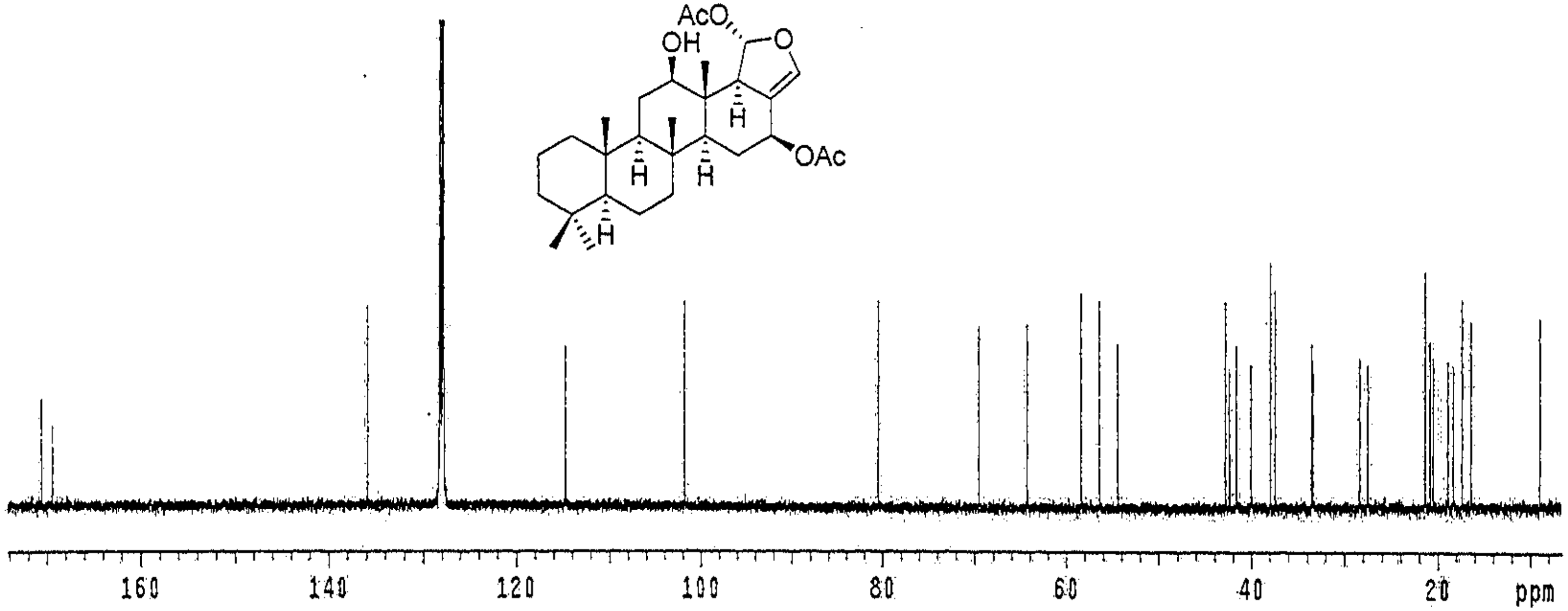


Figure 9 ^{13}C NMR spectrum of compound **1** (C_6D_6; 125 MHz)

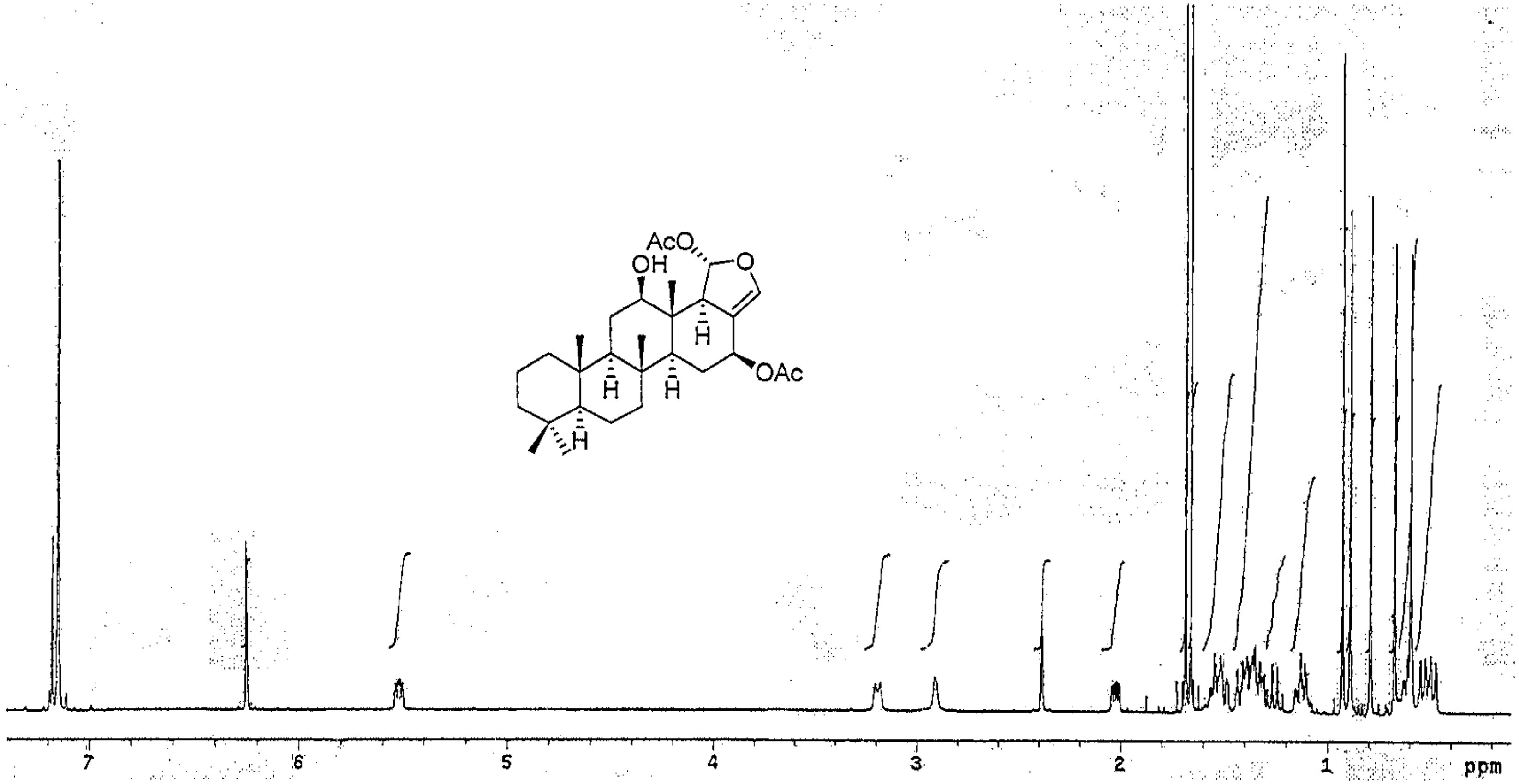
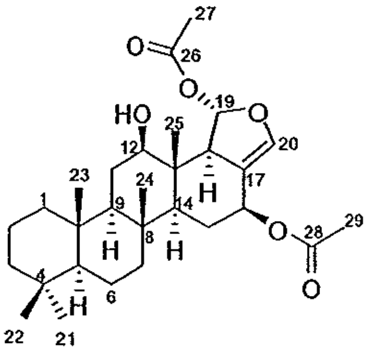


Figure 10 ^{1}H NMR spectrum of compound **1** (C_6D_6; 500 MHz)

one formerly seen in **46**. Similar HMBC correlations, i.e., from C-14 to H-7, H-15, H-16, H-18, H-24 and H-25, and from C-16 to H-20, H-18 and H-29, helped connecting rings A-D of **1**. The extension of C-17 to C-20 via an allylic coupling (J = 2.5 Hz) between H-16 and H-20 (δ 6.25) led to the connection toward ring E, which was composed of an acetal carbon at δ 101.8 (C-19), and a methine at δ 64.3 (C-18). By comparison of the ^{1}H and ^{13}C spectral of **1** with the previously reported data, compound **1** was proposed to be heteronemin (Kazlauskas *et al.*, 1976; Crews and Bescansa, 1986; Wonganuchitmeta *et al.*, 2004). The absolute configuration as shown was proposed after that in Kazlauskas *et al.* (1976).



1

Table 5 ^{1}H and ^{13}C NMR spectral data of compound **1** (C_6D_6; 500 MHz for ^{1}H)

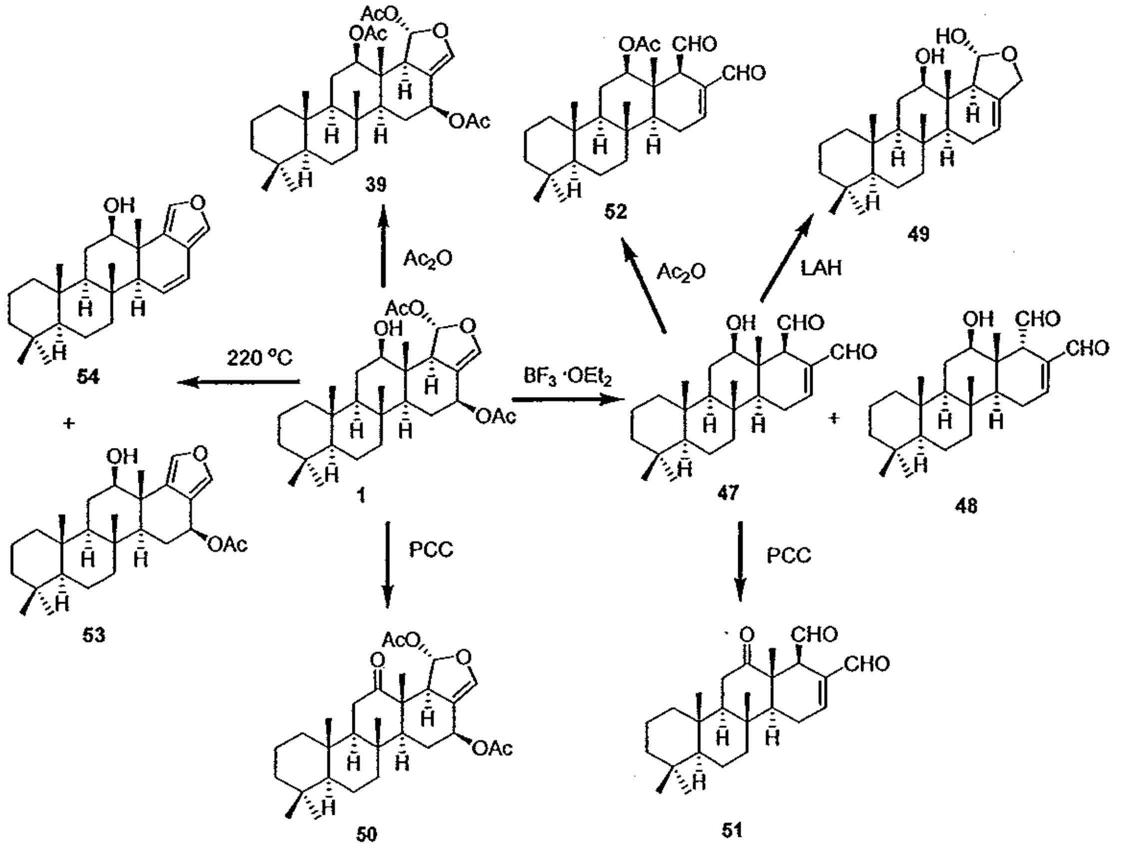
positions	^{1}H (mult.; J in Hz)	^{13}C (mult.)
1 eq	1.52 (m)	40.0 (CH_2)
ax	0.59 (m)	
2 eq	1.31 (m)	18.3 (CH_2)
ax	1.10 (m)	
3 eq	1.39 (m)	42.4 (CH_2)
ax	1.14 (m)	
4	-	33.3 (C)
5	0.63 (dd; 8.5, 3.5)	56.4 (CH)
6 eq	1.37 (m)	18.9 (CH_2)
ax	1.55 (m)	
7 eq	1.41 (m)	41.6 (CH_2)
ax	0.51 (m)	
8	-	37.4 (C)

Table 5 (cont.)

positions	^{1}H (mult.; *J* in Hz)	^{13}C (mult.)
9	0.49 (m)	58.4 (CH)
10	-	38.0 (C)
11 eq	1.50 (m)	27.5 (CH_2)
ax	1.25 (m)	
12	3.19 (dd; 11.5, 4.1)	80.5 (CH)
13	-	42.8 (C)
14	0.54 (dd; 11.0, 2.0)	54.4 (CH)
15 eq	2.03 (ddd; 12.0, 6.0, 2.0)	28.3 (CH_2)
ax	1.35 (m)	
16	5.12 (ddd; 10.5, 6.0, 2.5)	69.6 (CH)
17	-	114.7 (C)
18	2.39 (br s)	64.3 (CH)
19	7.18 (d; 1.5)	101.8 (CH)
20	6.25 (dd; 2.5, 1.5)	135.9 (CH)
21	0.89 (s; 3H)	33.5 (CH_3)
22	0.79 (s; 3H)	21.4 (CH_3)
23	0.67 (s; 3H)	16.4 (CH_3)
24	0.60 (s; 3H)	17.4 (CH_3)
25	0.93 (s; 3H)	9.0 (CH_3)
26	-	170.6 (C)
27	1.67 (s; 3H)	20.8 (CH_3)
28	-	169.4 (C)
29	1.69 (s; 3H)	20.5 (CH_3)
12-OH	2.91 (br s)	-

3.2 Chemical derivatization of marine-derived scalaranes

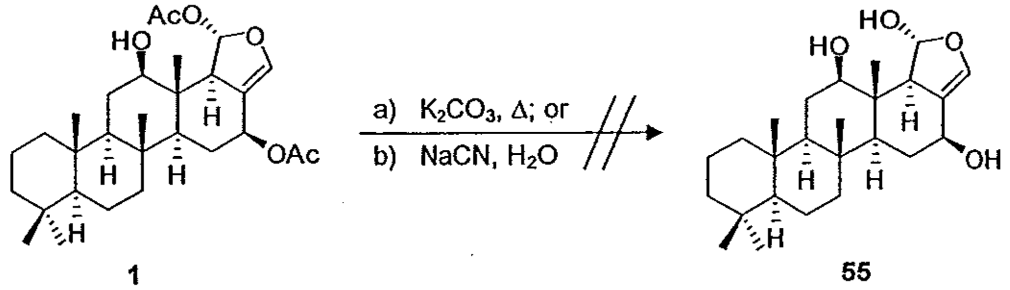
As postulated by Crews and Bescansa (1986), the activity of heteronemin (1) was influenced by the oxygenation pattern surrounding rings C, D, and E. In order to extend the chemical library and to establish an activity profile of the scalarins, a series of scalarin derivatives were synthesized using heteronemin as starting material. Primary transformations were focused on the furan moiety and the oxygenated functionalities on C-12 and C-16. All the transformations are summarized in Scheme **1**.



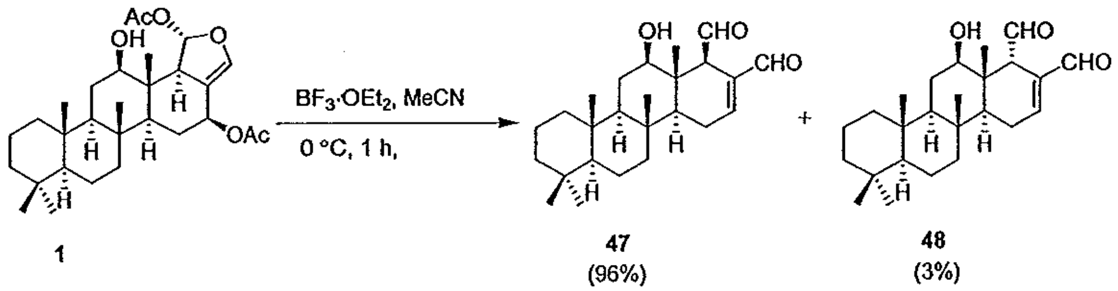
Scheme 1 Summarized chemical transformations of scalarin derivatives.

3.2.1 Hydrolytic cleavage of furanol acetate

Among the first attempts to modify the chemical structure of heteronemin was the manipulation of the acetoxy groups on C-16 and C-19, namely towards a more easily transformable hydroxyl ones. Unfortunately, the acetal moiety of **1** proved to be too labile to most chemical transformation, including saponification and transesterification (Scheme 2). Surprisingly, the compound however cleanly underwent the hydrolytic cleavage with $BF_3 \cdot OEt_2$ in wet MeCN (Askin *et al.*, 1987), and the dialdehyde **47** (96%) was obtained, along with the epimeric **48** (3%) as traced minor product (Scheme 3). Due to the cleanliness and high yield, **1** and **47** were hereafter used as major platforms for the further transformations.

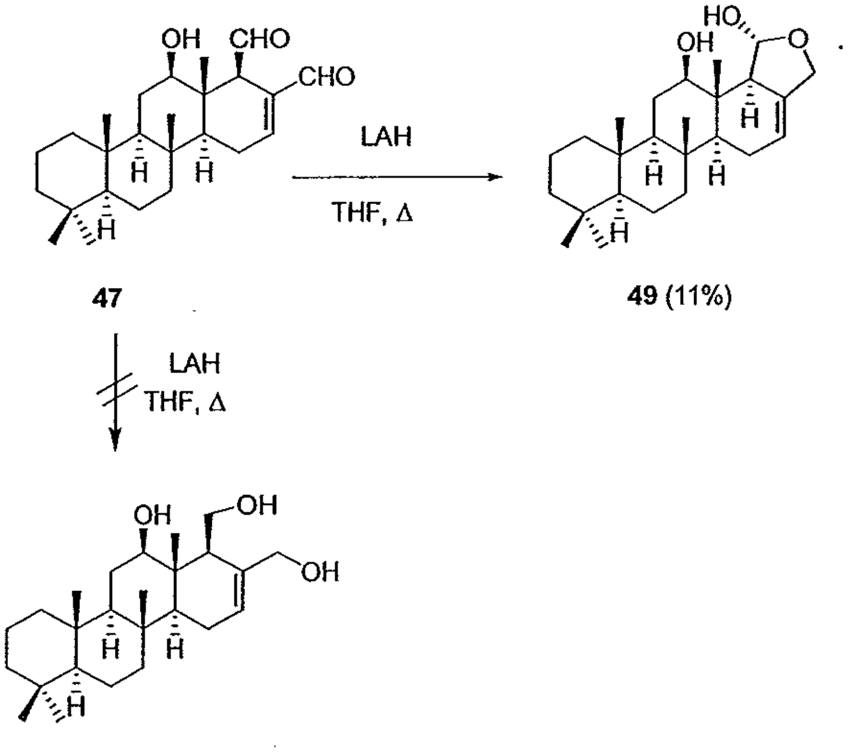


Scheme 2 Saponification and transesterification of heteronemin



Scheme 3 Hydrolytic cleavage of 1

To manipulate the polarily of the dialdehyde 47, hydride reduction with LAH was carried out (Kuehne *et al.*, 1988). Instead of the expected triol (56), however, a re-cyclized lactol 49 was obtained (11%; Scheme 4). The configuration of the furanol moiety was proved by means of nOe, as expected to be dictated by Cram's rule.



47

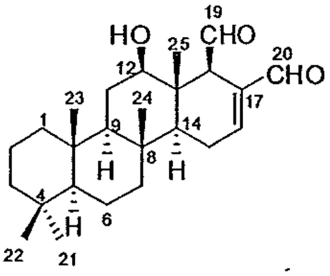
49 (11%)

56

Scheme 4 Reduction of 47

3.2.1.1 12-Deacetyl-12-*epi*-scalaradial (47)

Compound 47, was isolated as white solid from the hydrolytic cleavage of **1** with $BF_3 \cdot OEt_2$. The HREIMS indicated a formula of $C_{25}H_{38}O_3$ according to the molecular ion peak at *m/z* 386.2818 $[M]^+$ (calcd for $C_{25}H_{38}O_3$, 386.2821). As expected, all the resonances in the NMR spectra (Table 6) related to the acetate groups formerly seen in the spectra of **1** were entirely removed. In fact, along with the acetate functionalities, the resonances for the furan moiety were completely replaced by two aldehydes (δ_H 10.02, d; J = 3.0 Hz, H-19; and 9.15, br s, H-20; and δ_C 202.7, C-19; and 192.2, C-20). The aldehydic carbon C-20, in particular, also correlated to an olefin at δ 151.3 (C-16). These indicated the cleavage of furan moiety of **1** into two aldehydes attached to C-17 and C-18. From the NOSEY correlations, the aldehydic proton H-19 showed the dipolar coupling to the methyl protons at δ 0.74 (s, 3H; H-25), and the proton at δ 3.00 (br s, H-18) correlated to the methine protons of H-12 (δ 3.28, dd; J = 11.5, 4.0 Hz) and H-14 (δ 0.57, dd; J = 11.5, 5.0 Hz), suggesting that C-19, C-25 and 12-OH adopted the *β* orientation, whereas H-12, H-14, and H-18 did the *α* one. Concurred to the previously reported spectral data (Crews and Bescansa, 1986), the structure of **47** was confirmed to be 12-deacetyl-12-*epi*-scalaradial.



47

Table 6 ^{1}H and ^{13}C NMR data of compound **47** (C_6D_6; 500 MHz for ^{1}H)

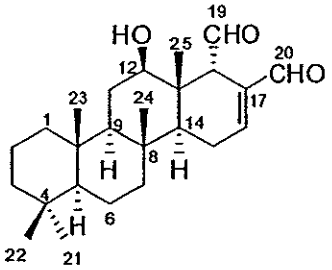
positions	^{1}H (mult.; *J* in Hz)	^{13}C (mult.)
1 eq	1.56 (m)	40.0 (CH_2)
ax	0.63 (m)	
2 eq	1.38 (m)	18.3 (CH_2)
ax	1.12 (m)	
3 eq	1.34 (m)	41.3 (CH_2)
ax	1.31 (m)	
4	-	33.3 (C)
5	0.65 (dd; 8.5, 2.0)	56.3 (CH)
6 eq	1.57 (m)	18.8 (CH_2)
ax	1.53 (m)	
7 eq	1.35 (m)	42.4 (CH_2)
ax	1.12 (ddd; 12.5, 7.0, 3.5)	
8	-	37.4 (C)
9	0.47 (br d; 12.5)	57.9 (CH)
10	-	37.4 (CH)
11 eq	1.43 (ddd; 13.0, 4.0, 3.5)	27.3 (CH_2)
ax	1.15 (ddd; 13.0, 11.5, 7.5)	
12	3.28 (dd; 11.5, 4.0)	80.8 (CH)
13	-	43.7 (C)

Table 6 (cont.)

positions	^{1}H (mult.; *J* in Hz)	^{13}C (mult.)
14	0.57 (dd; 11.5, 5.0)	52.9 (CH)
15 eq	1.68 (ddd; 14.0, 5.0, 2.0)	23.4 (CH_2)
ax	1.72 (ddd; 14.0, 11.5, 2.5)	
16	6.14 (dd; 5.0, 2.5)	151.3 (CH)
17	-	139.8 (C)
18	3.00 (br s)	60.7 (CH)
19	10.02 (d; 3.0)	202.7 (CH)
20	9.15 (s)	192.2 (CH)
21	0.87 (s; 3H)	33.5 (CH_3)
22	0.80 (s; 3H)	21.4 (CH_3)
23	0.69 (s; 3H)	16.6 (CH_3)
24	0.61 (s; 3H)	17.1 (CH_3)
25	0.74 (s; 3H)	10.0 (CH_3)

3.2.1.2 12-Deacetyl-12,18-di-*epi*-scalaradial (48)

Compound 48 was isolated as a minor products from hydrolytic cleavage of 1 with $BF_3 \cdot OEt_2$. The HREIRMS spectrum showing the signal at *m/z* 386.2803 (calcd for $C_{25}H_{38}O_3$, 386.2821, $[M]^+$) revealed a molecular formula of $C_{25}H_{38}O_3$. The ^{1}H and ^{13}C NMR spectra of 48 (Table 7) were entirely identical to 47, indicated that the two compounds are in fact stereoisomers. The major difference observable as the downfield shift of H-14 (δ 1.29, dd; J = 11.0, 5.5 Hz), as compared to that resonating at δ 0.57 for 47, was accounted for the anisotropic effect posted on H-14 by the aldehyde carbonyl. The relative stereochemistry of the aldehyde C-19 was determined to be α-oriented, based on the nOe enhancement at H-19 (δ 10.00, d; J = 2.5 Hz) upon the irradiation of H-12 (δ 3.31, dd; J = 11.0, 4.0 Hz). The structure of 48 was therefore proposed to be 12-deacetyl-12,18-di-*epi*-scalaradial (Walker *et al.*, 1980).



48

Table 7 ^{1}H and ^{13}C NMR data of compound 48 (C_6D_6; 500 MHz for ^{1}H)

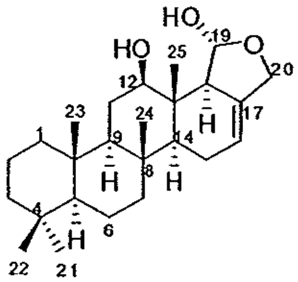
positions	^{1}H (mult.; *J* in Hz)	^{13}C (mult.)
1 eq	1.46 (br d; 13.5)	39.8 (CH_2)
ax	0.56 m	
2 eq	1.55 (qt; 13.5, 4.0)	18.3 (CH_2)
ax	1.37 (m)	
3 eq	1.36 (m)	42.1 (CH_2)
ax	1.11 (m)	
4	-	33.3 (C)
5	0.53 (dd; 9.5, 2.5)	55.9 (CH)
6 eq	1.35 (m)	18.2 (CH_2)
ax	1.15 (m)	
7 eq	1.33 (m)	41.0 (CH_2)
ax	0.57 (m)	
8	-	37.3 (C)
9	0.50 (dd; 9.5, 2.0)	58.0 (CH)
10	-	37.4 (C)
11 eq	1.26 (m)	27.0 (CH_2)
ax	1.18 (m)	
12	3.31 (dd; 11.0, 4.0)	75.5 (C)
13	-	43.5 (C)
14	1.29 (dd; 11.0, 5.5)	48.0 (CH)
15 eq	1.90 (dt; 20.5, 5.5)	23.9 (CH_2)
ax	1.65 (m)	
16	6.21 (dd; 5.0, 2.5)	151.2 (CH)

Table 7 (cont.)

positions	^{1}H (mult.; *J* in Hz)	^{13}C (mult.)
17	-	137.9 (C)
18	3.89 (br s)	55.3 (CH)
19	10.00 (d; 2.5)	202.0 (CH)
20	9.21 (s)	192.0 (CH)
21	0.86 (s; 3H)	33.3 (CH_3)
22	0.80 (s; 3H)	21.5 (CH_3)
23	0.70 (s; 3H)	16.9 (CH_3)
24	0.59 (s; 3H)	16.8 (CH_3)
25	0.68 (s; 3H)	15.9 (CH_3)

3.2.1.3 12-Deacetyl-12-*epi*-deoxoscalarin (49)

Compound 49, was obtained as a reductive recyclized product from the reduction of 1 with LAH (11%). The compound showed a pseudo-molecular ion peak at *m/z* 411.2866 $[M+Na]^+$ in the HRESIMS (calcd for $C_{25}H_{40}O_3Na$, 411.2875); therefore a molecular formula of $C_{25}H_{40}O_3$ was proposed. The ^{1}H and ^{13}C NMR spectra of 49 (Table 8) differed from 1 by the absence of two acetate groups and the presence of the oxymethylene protons at δ 4.50 (br d; J = 12.0 Hz, H-20a) and 4.18 (br d; J = 12.0 Hz, H-20b). In addition, an olefinic proton signal at δ 5.48 (br s, H-16) of 49 was found at the higher field than that of 1 due to the double bond migration away from the electronegative oxygen. For the furan moiety, the reduction led to the recyclization towards a tetrahydrofuranol functionality, composed of an acetal proton at δ 5.25 (d; J = 6.5 Hz, H-19), an oxymethylene at δ 4.50 (br d; J = 12.0 Hz, H-20a) and 4.18 (br d; J = 12.0 Hz, H-20b), and a methine at δ 2.19 (br s, H-18). The structure of 49 was proposed to be 12-deacetyl-12-*epi*-deoxoscalarin, and was confirmed by comparison with the published data (Fontana *et al.*, 1999).



49

Table 8 ^{1}H and ^{13}C NMR data of compound **49** (C_6D_6; 500 MHz for ^{1}H)

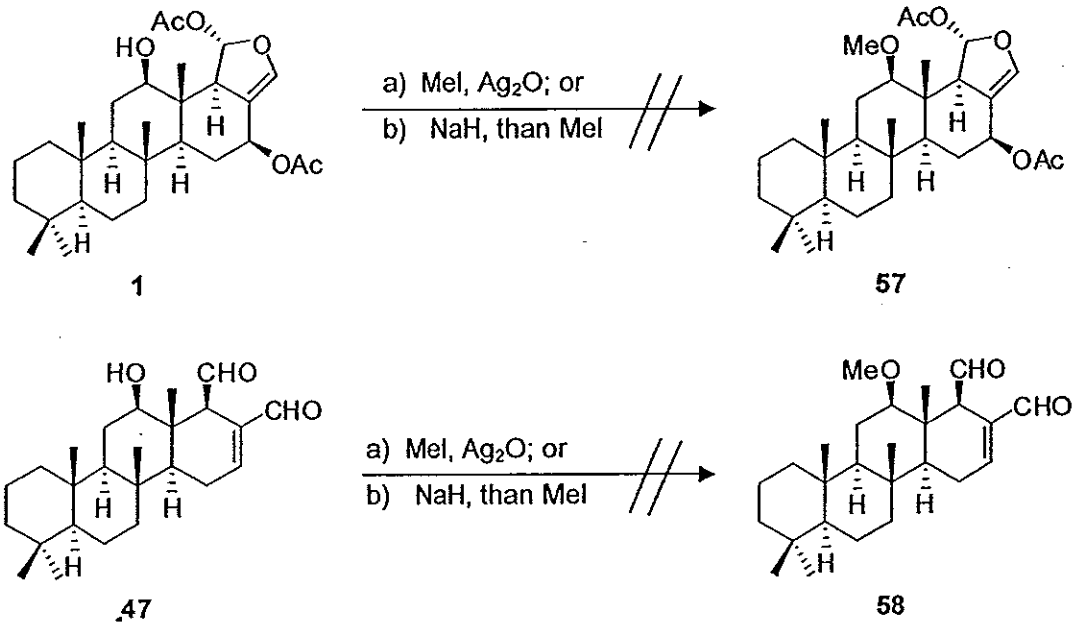
positions	^{1}H (mult.; *J* in Hz)	^{13}C (mult.)
1 eq	1.70 (m)	39.9 (CH_2)
ax	0.78 (m)	
2 eq	1.50 (m)	18.1 (CH_2)
ax	1.33 (m)	
3 eq	1.66 (m)	41.6 (CH_2)
ax	0.85 (m)	
4	-	33.3 (C)
5	0.77 (dd; 11.0, 2.5)	56.5 (CH)
6 eq	1.58 (m)	18.6 (CH_2)
ax	1.38 (m)	
7 eq	1.34 (m)	42.1 (CH_2)
ax	1.11 (m)	
8	-	37.4 (C)
9	0.87 (m)	58.8 (CH)
10	-	37.4 (C)
11 eq	1.69 (m)	25.8 (CH_2)
ax	1.42 (q; 12.0)	
12	3.50 (dd; 12.0, 4.0)	81.2 (CH)
13	-	39.9 (C)
14	1.00 (dd; 11.0, 6.0)	53.3 (CH)
15 eq	2.04 (m)	22.1 (CH_2)
ax	1.98 (m)	
16	5.48 (br s)	117.2 (CH)
17	-	135.0 (C)

Table 8 (cont.)

positions	^{1}H (mult.; *J* in Hz)	^{13}C (mult.)
18	2.19 (br s)	60.8 (CH)
19	5.25 (d; 6.5)	99.5 (CH)
20	4.50 (br d; 12.0)	68.7 (CH_2)
	4.18 (br d; 12.0)	
21	0.82 (s; 3H)	33.4 (CH_3)
22	0.78 (s; 3H)	21.3 (CH_3)
23	0.89 (s; 3H)	16.5 (CH_3)
24	0.90 (s; 3H)	17.0 (CH_3)
25	0.81 (s; 3H)	8.7 (CH_3)

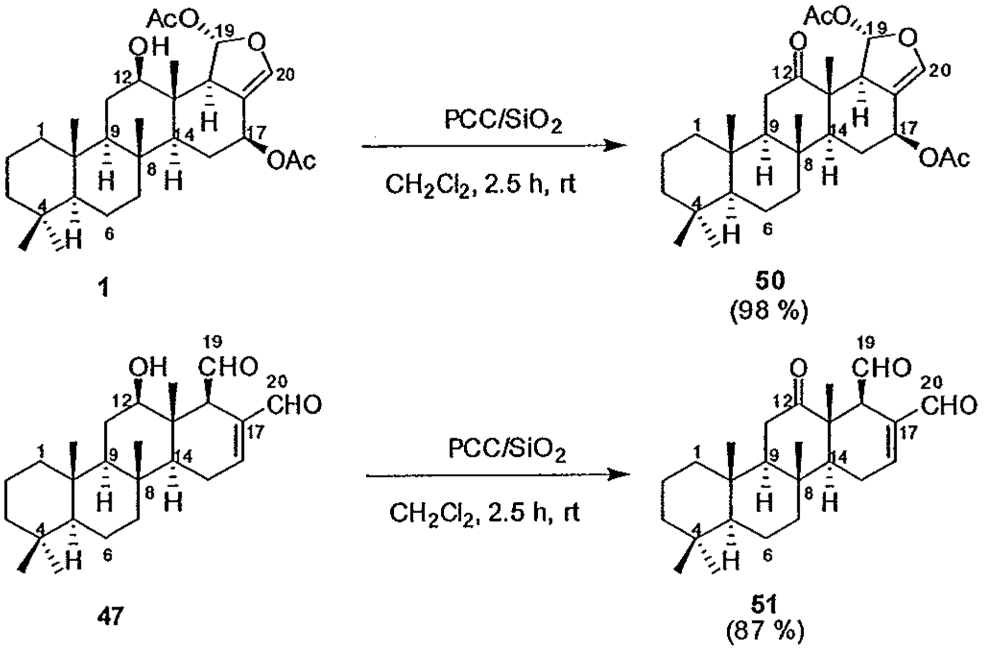
3.2.2 Modification of 12-OH functionality

In order to manipulate the electronic field surrounding C-12, the hydroxyl group was planned to be transformed into oxo, alkoxyl and acyloxy functionalities. Methylation based on Williamson's ether synthesis (Benedict *et al.*, 1979), however, failed to yield the expected methoxy analogues, either from **1** or from **47** (Scheme 5). This is possibly due to the steric hindrance surrounding C-12, providing an environment similar to a neopentyl system, thus retarding the substitution onto MeI.



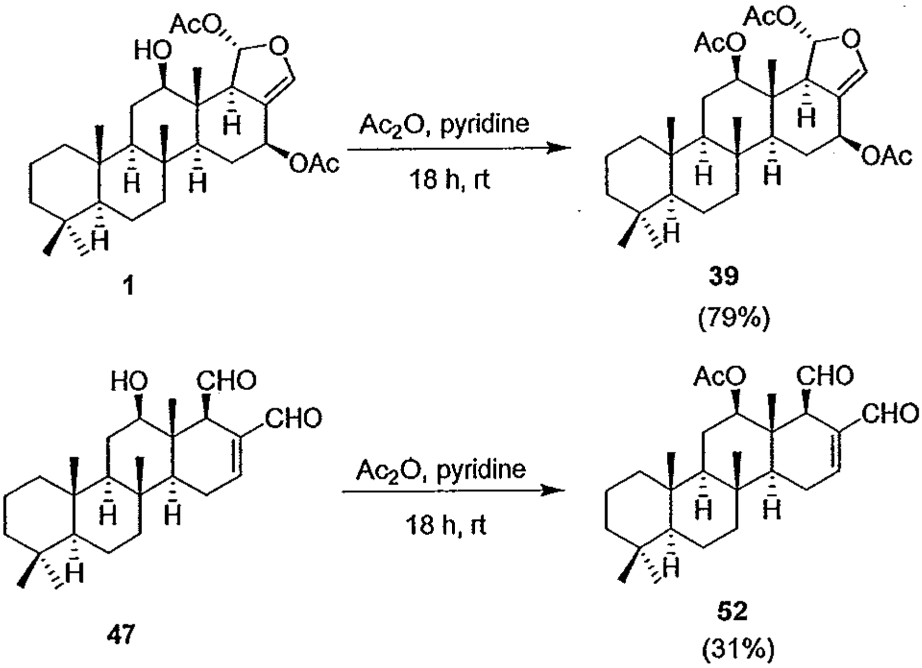
Scheme 5 Methylation of **1** and **47**

On the other hand, PCC surprisingly oxidized the C-12 hydroxy group and provided ketones, both from heteronemin itself and from the dial 47, without observable hydrolysis or epimerization (Adams and Luzzio, 1989). The oxo 50 was obtained from the reaction of 1 with PCC in CH_2Cl_2 in a good yield (98%). Likewise, the dial 47 was oxidized smoothly to yield the oxo-dial 51 (87%) (Scheme 6).



Scheme 6 Oxidation of 1 and 47

Unlike the sluggish alkylation, acetylation of 1 was accomplished using a standard acetylation condition to gave heteronemin acetate 39 in a reasonable yield (79%). The acetylation of dial 47 was carried out using the similar protocol to give compound 52 as the acetate analog of 47 in a lower yield (31%) (Scheme 7).

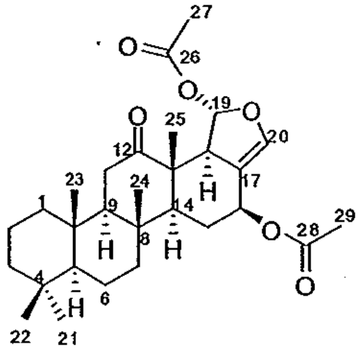


Scheme 7 Acetylation of 1 and 47

3.2.2.1 12-Oxoheteronemin (50)

Compound 50 was obtained from an oxidation of 1 with PCC (98%). The molecular formula of 50 was proposed to be $C_{27}H_{38}O_4$ as established by the EI mass spectrum, which shows a molecular peak at *m/z* 426 $[M]^+$, and by its 27 carbon signals observed from the ^{13}C NMR spectrum. The proposed molecular formula was confirmed by the $[M]^+$ peak at *m/z* 426.2788 in the high resolution ESI mass spectrum (calcd for $C_{27}H_{38}O_4$, 426.2770).

The ^1H and ^{13}C NMR spectral data (Table 9) resembled those of 1, except that the signal of the oxymethine carbon of 1 at δ 80.5, previously assigned to C-12, was replaced by a carbonyl carbon at δ 213.3. Also, the downfield shift of H-11 (δ 2.12, dd; J = 14.0, 2.5 Hz; and 2.05, t; J = 14.0 Hz) due to the anisotropic effect from the carbonyl was observed. The assignment of the carbonyl as C-12 was supported by the HMBC spectrum, in which the correlations from C-12 to H-9, H-18, H-11 and H-25 were found. Thus, the structure of 50 was assigned as 12-oxoheteronemin as shown (Crews and Bescansa, 1986).



50

Table 9 ^{1}H and ^{13}C NMR data of compound **50** (C_6D_6; 500 MHz for ^{1}H)

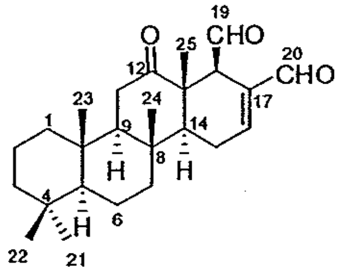
positions	^{1}H (mult.; J in Hz)	^{13}C (mult.)
1 eq	1.29 (m)	39.2 (CH_2)
ax	0.52 (m)	
2 eq	1.41 (m)	18.0 (CH_2)
ax	1.25 (m)	
3 eq	1.31 (m)	42.0 (CH_2)
ax	1.00 (m)	
4	-	33.2 (C)
5	0.46 (dd; 12.0, 2.5)	55.9 (CH)
6 eq	1.23 (m)	18.6 (CH_2)
ax	1.04 (m)	
7 eq	1.37 (m)	41.1 (CH_2)
ax	1.28 (m)	
8	-	37.7 (C)
9	0.69 (dd; 14.0, 2.5)	54.4 (CH)
10	-	37.9 (C)
11 eq	2.12 (dd; 14.0, 2.5)	35.1 (CH_2)
ax	2.05 (t; 14.0)	
12	-	213.1 (C)
13	-	50.0 (C)
14	0.85 (dd; 12.0, 2.5)	56.0 (CH)
15 eq	1.95 (dt; 6.5, 2.5)	27.8 (CH_2)
ax	1.23 (m)	

Table 9 (cont.)

positions	^{1}H (mult.; *J* in Hz)	^{13}C (mult.)
16	5.43 (m)	69.2 (CH)
17	-	113.0 (C)
18	3.06 (d; 1.0)	56.8 (CH)
19	7.11 (d; 2.0)	99.4 (CH)
20	6.26 (dd; 2.19, 1.75)	137.0 (CH)
21	0.84 (s; 3H)	33.3 (CH_3)
22	0.73 (s; 3H)	21.3 (CH_3)
23	0.53 (s; 3H)	15.3 (CH_3)
24	0.55 (s; 3H)	16.6 (CH_3)
25	0.76 (s; 3H)	13.1 (CH_3)
26	-	169.3 (C)
27	1.69 (s; 3H)	20.5 (CH_3)
28	-	169.3 (C)
29	1.78 (s; 3H)	20.7 (CH_3)

3.3.2.2 12-Deacetoxy-12-oxoscalaradial (51)

Compound 51 was obtained as an oxidative product of 47 with PCC. The molecular formula of $C_{25}H_{36}O_3$ was inferred by means of HREIMS spectrum (*m/z* 384.2668 $[M]^+$; calcd for $C_{25}H_{36}O_3$, 384.2664). The 2 mass unit less that of 47 suggested that 51 was a dehydro-product of 47. The similarity between the NMR spectra of 47 (Table 6) and 51, with the absence of an oxymethine C-12 (δ 80.8) formerly seen in 47, and the presence of a carbonyl (δ 212.6, C-12) in 51, suggested that the oxidation took place on the hydroxyl group on C-12. This was confirmed as a series of HMBC correlations from C-12 to H-9, H-11, and H-25 were observed. Compound 51 was therefore proposed as 12-deacetoxy-12-oxoscalaradial (Gavagnin *et al.*, 2004).



51

Table 10 ^{1}H and ^{13}C NMR data of compound **51** (C_6D_6; 500 MHz for ^{1}H)

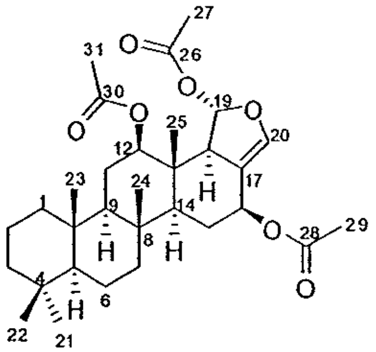
positions	^{1}H (mult.; *J* in Hz)	^{13}C (mult.)
1 eq	1.31 (m)	39.2 (CH_2)
ax	0.39 (m)	
2 eq	1.45 (m)	18.0 (CH_2)
ax	1.21 (m)	
3 eq	1.38 (m)	42.0 (CH_2)
ax	1.01 (m)	
4	-	33.4 (C)
5	0.51 (m)	56.1 (CH)
6 eq	1.47 (m)	18.5 (CH_2)
ax	1.17 (m)	
7 eq	1.26 (m)	41.0 (CH_2)
ax	1.12 (m)	
8	-	37.1 (C)
9	0.72 (dd; 11.4, 2.1)	58.5 (CH)
10	-	37.7 (C)
11 eq	2.18 (dd; 4.2, 2.0)	35.0 (CH_2)
ax	2.00 (t; 4.1)	
12	-	212.6 (C)
13	-	53.3 (C)
14	0.85 (m)	54.6 (CH)
15 eq	1.72 (m)	23.1 (CH_2)
ax	1.58 (m)	
16	6.02 (br t; 2.7)	148.8 (CH)
17	-	142.0 (C)
18	3.70 (br d; 1.7)	53.9 (CH)

Table 10 (cont.)

positions	^{1}H (mult.; *J* in Hz)	^{13}C (mult.)
19	10.76 (d; 1.9)	200.2 (CH)
20	9.14 (s)	191.5 (CH)
21	0.83 (s; 3H)	33.2 (CH_3)
22	0.75 (s; 3H)	21.4 (CH_3)
23	0.54 (s; 3H)	15.6 (CH_3)
24	0.58 (s; 3H)	16.2 (CH_3)
25	0.87 (s; 3H)	15.1 (CH_3)

3.2.2.3 Heteronemin acetate (39)

Acetylation of **1** using Ac_2O in pyridine afforded compound 39 in a good yield (79%). The molecular formula of compound 39 was deduced to be $C_{31}H_{46}O_7$ by means of the analysis of its HRESI mass spectrum, which showed pseudo-molecular ion peak at *m/z* 553.3121 ($[M+Na]^+$; calcd for $C_{31}H_{46}O_7Na$, 553.3142). The 43 mass units higher than that of 1 suggested the additional acetyl group. The ^{1}H and ^{13}C NMR spectra (Table 11) of 39 displayed characteristic resonances similar to those of 1, however, with an additional methyl group at δ 1.97 (s, H-31). The ^{13}C NMR spectra indicated the presence of an ester carbonyl carbon at δ 169.9 (C-30). The connectivity of the added acetate was confirmed by the HMBC spectrum as the correlation from C-12 (δ 82.2) to H-31 (1.97) was observed. The nOe enhancement of H-14 (δ 0.58) and H-18 (δ 2.59) upon irradiation of H-12 indicated the conserved configuration of C-12 as *R*. Therefore, compound 39 was identified as heteronemin acetate. The spectral data of 39 are consistent with those previously reported (Crews and Bescansa, 1986).



39

Table 11 ^{1}H and ^{13}C NMR data of compound 39 (C_6D_6; 500 MHz for ^{1}H)

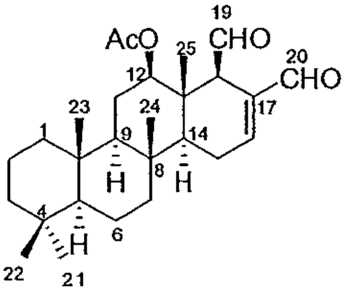
positions	^{1}H (mult.; *J* in Hz)	^{13}C (mult.)
1 eq	1.37 (m)	39.7 (CH_2)
ax	0.71 (m)	
2 eq	1.34 (m)	18.2 (CH_2)
ax	1.17 (m)	
3 eq	1.32 (m)	42.2 (CH_2)
ax	1.17 (m)	
4	-	33.3 (C)
5	0.61 (m)	56.1 (CH)
6 eq	1.48 (m)	18.8 (CH_2)
ax	1.28 (m)	
7 eq	1.46 (m)	41.4 (CH_2)
ax	0.47 (m)	
8	-	37.4 (C)
9	0.55 (m)	57.6 (CH)
10	-	37.8 (C)
11 eq	1.67 (m)	24.0 (CH_2)
ax	1.22 (m)	
12	4.67 (dd; 11.2, 4.4)	82.2 (CH)
13	-	40.8 (C)
14	0.58	54.6 (CH)
15 eq	2.00 (ddd; 12.0, 7.5, 2.0)	27.8 (CH_2)
ax	1.25 (m)	
16	5.48 (dd; 11.0, 6.0)	69.3 (CH)
17	-	113.6 (C)
18	2.59 (br s)	62.3 (CH)
19	7.08 (d; 2.0)	98.8 (CH)
20	6.21 (t; 2.0)	136.2 (CH)
21	0.87 (s; 3H)	33.4 (CH_3)
22	0.76 (s; 3H)	21.3 (CH_3)
23	0.62 (s; 3H)	16.4 (CH_3)
24	0.57 (s; 3H)	17.3 (CH_3)

Table 11 (cont.)

positions	^1H (mult.; *J* in Hz)	^{13}C (mult.)
25	0.93 (s; 3H)	10.0 (CH_3)
26	-	169.7 (C)
27	1.70 (s; 3H)	20.6 (CH_3)
28	-	169.3 (C)
29	1.69 (s; 3H)	20.5 (CH_3)
30	-	169.9 (C)
31	1.97 (s; 3H)	21.1 (CH_3)

3.2.3.4 12-*epi*-Scalaradial (52)

Acetylation of 47 using Ac_2O led to compound 52 as white solid (31%, Gavagnin *et al.*, 2004). The molecular formula of 52 was proposed as $C_{27}H_{40}O_4$ due to the molecular mass observed at *m/z* 451.2837 in HREIMS spectrum (calcd. for $C_{27}H_{40}O_4$, 428.2527). The ^1H and ^{13}C NMR spectra (Table 12) of 52 displayed characteristic signals similar to those of 51, except for the presence of an additional methyl group at δ_H 1.78 (s, 3H, H-27) in 52, which correlated to a carbon at δ 169.9 (C-26) in the HMBC spectrum. This additional acetate group was placed on C-12 due to its HMBC spectrum, in which C-12 (δ 81.7) showed correlation to the protons at δ 1.85 (ddd; J = 13.0, 4.5, 2.0 Hz, H-11eq), 1.15 (ddd; J = 13.0, 12.0, 12.0 Hz, H-11ax), 1.78 (s, H-27) and 0.85 (s, H-25). The relative stereochemistry of hydroxyl and aldehyde groups was determined based on the nOe enhancement. Irradiation of H-12 caused the signal enhancement of H-14 and H-18, indicating the β-oriented of the 12-acetoxy and the carboxaldehyde C-19. Thus, the structure 52 was deduced to be 12-*epi*-scalaradial (Crews and Bescansa, 1986).



52

Table 12 ^{1}H and ^{13}C NMR data of compound 52 (C_6D_6; 500 MHz for ^{1}H)

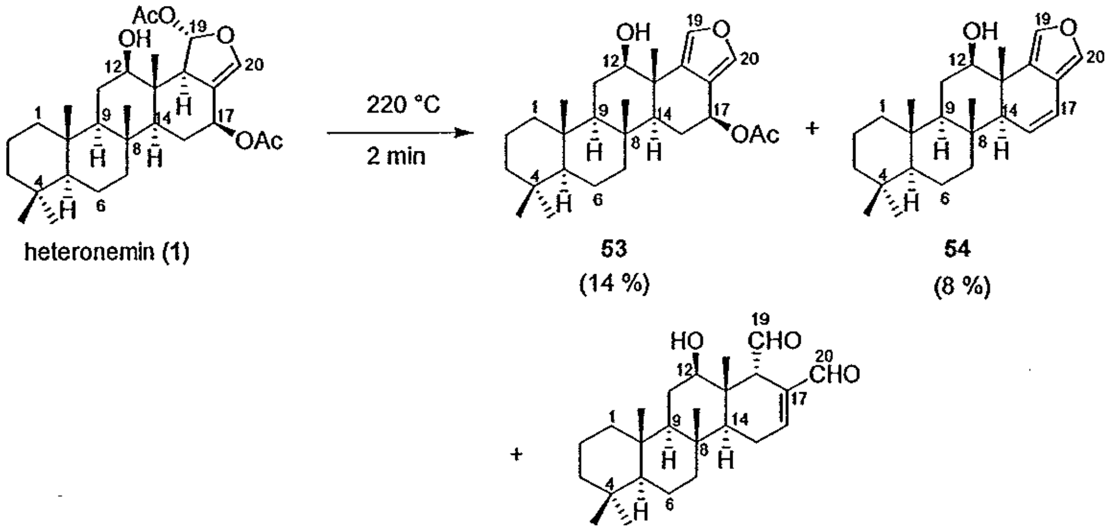
positions	^{1}H (mult.; *J* in Hz)	^{13}C (mult.)
1 eq	1.51 (m)	39.6 (CH_2)
ax	0.75 (m)	
2 eq	1.47 (m)	18.7 (CH_2)
ax	1.35 (m)	
3 eq	1.32 (m)	41.2 (CH_2)
ax	1.08 (m)	
4	-	33.4 (C)
5	0.62 (dd; 12.0, 2.5)	56.2 (CH)
6 eq	1.39 (m)	18.2 (CH_2)
ax	1.10 (m)	
7 eq	1.28 (m)	42.0 (CH_2)
ax	0.45 (ddd; 16.5, 11.5, 3.5)	
8	-	37.3 (C)
9	0.69 (m)	57.2 (CH)
10	-	37.4 (C)
11 eq	1.85 (ddd; 13.0, 4.5, 2.0)	23.7 (CH_2)
ax	1.15 (ddd; 13.0, 12.0, 12.0)	
12	4.75 (dd; 12.0, 4.5)	81.7 (CH)
13	-	41.1 (C)
14	0.61 (m)	52.8 (CH)
15 eq	1.68 (ddd; 15.5, 4.0, 3.5)	23.3 (CH_2)
ax	1.65 (ddd; 15.5, 12.0, 3.0)	
16	6.05 (dd; 4.0, 3.0)	151.2 (CH)
17	-	139.4 (C)
18	2.97 (br d; 2.0)	60.2 (CH)
19	9.85 (d; 4.0)	199.0 (CH)
20	9.09 (s)	191.7 (CH)
21	0.87 (s; 3H)	33.3 (CH_3)
22	0.78 (s; 3H)	21.4 (CH_3)
23	0.65 (s; 3H)	16.6 (CH_3)

Table 12 (cont.)

positions	^{1}H (mult.; *J* in Hz)	^{13}C (mult.)
24	0.61 (s; 3H)	16.9 (CH_3)
25	0.85 (s; 3H)	11.0 (CH_3)
26	-	169.6 (C)
27	1.78 (s; 3H)	20.9 (CH_3)

3.2.3 Pyrolysis of heteronemin

All the transformation discussed earlier mostly involve the lost of the acetoxyl groups either, or both, on C-17 and C-19. In order to extend the chemical profile of the scalarins in such way that the oxidation state and saturation of the furan moiety could be manipulated, compound **1** was subjected to a pyrolytic conditions under an inert atmosphere of N_2. As predicted, **1** was heat-labile and large portion of the compound degraded promptly upon only quick exposure to the high temperature (220 °C). Two pyrolytic products obtained were the furan **53** and the vinyl furan **54** analogues of heteronemin along with a cleavage product **48**.



heteronemin (1)

53
(14 %)

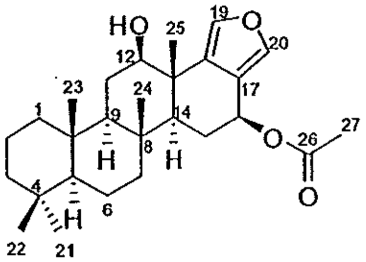
54
(8 %)

48
(17%)

Scheme 8 Pyrolysis of heteronemin

3.2.3.1 Scalarafuran (53)

Compound 53 was obtained as a pyrolytic product of heteronemin (14%). The HREIMS mass measurement at *m/z* 368.2710 (calcd for $C_{25}H_{36}O_2$, 368.2706) indicated the molecular formula at $C_{25}H_{36}O_2$. The UV absorptions at λ_{max} 208, 218 and 273 nm, suggested the presence of an aromatic chromophore in the molecule. The ^1H and ^{13}C NMR spectral data (Table 13) of 53 were comparable to those of 1 with the disappearance of dioxymethine H-19, a methine H-18, and the acetate of H-16 (δ 5.97, dd; J = 12.0, 2.0 Hz). On the other hand, additional resonances of furan moiety at δ 7.80 (d; J = 2.0 Hz, H-19) and 7.48 (t; J = 1.5 Hz, H-20) were found. The HMBC correlation from both C-17 (δ 135.3) and C-18 (δ 121.8) to H-19 and H-20 helped connecting the furan onto ring D, substituting the furanol moiety formerly seen in 1. Compound 53, therefore, was proposed as scalarafuran (Kazlauskas *et al.*, 1976).



53

Table 13 ^1H and ^{13}C NMR data of compound 53 (C_6D_6; 500 MHz for ^1H)

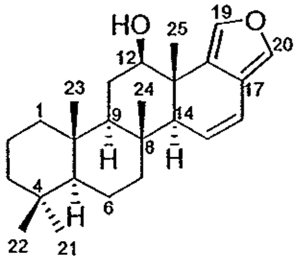
positions	^1H (mult.; J in Hz)	^{13}C (mult.)
1 eq	1.51 (m)	39.9 (CH_2)
ax	0.49 (m)	
2 eq	1.60 (m)	18.3 (CH_2)
ax	1.40 (m)	
3 eq	1.53 (m)	41.4(CH_2)
ax	0.53 (m)	
4	-	33.3 (C)
5	0.60 (dd; 12.5, 2.0)	56.4(CH)

Table 13 (cont.)

positions	^{1}H (mult.; *J* in Hz)	^{13}C (mult.)
6 eq	1.68 (m)	18.8 (CH_2)
ax	1.43 (m)	
7 eq	1.35 (m)	42.4(CH_2)
ax	1.11 (m)	
8	-	37.3 (C)
9	0.85 (m)	53.8 (CH)
10	-	37.5 (C)
11 eq	1.32 (m)	27.8 (CH_2)
ax	1.14 (m)	
12	3.27 (br d; 11.0)	79.2 (CH)
13	-	40.4 (C)
14	0.49 (dd; 12.5, 2.0)	58.1 (CH)
15 eq	2.09 (ddd; 12.0, 7.5, 2.0)	25.0 (CH_2)
ax	1.18 (m)	
16	5.97 (dd; 12.0, 2.0)	68.3 (CH)
17	-	135.3 (C)
18	-	121.8 (C)
19	7.80 (d; 2.0)	137.8 (CH)
20	7.48 (t; 1.5)	139.6 (CH)
21	0.90 (s; 3H)	33.5 (CH_3)
22	0.82 (s; 3H)	21.4 (CH_3)
23	0.71 (s; 3H)	17.5 (CH_3)
24	0.67 (s; 3H)	16.3 (CH_3)
25	1.18 (s; 3H)	18.9 (CH_3)
26	-	170.5 (C)
27	1.67 (s; 3H)	20.9 (CH_3)

3.2.3.2 16-Deacetoxy-15,16-dehydroscalarafuran (54)

Compound **54** was obtained as another pyrolytic product along with **53**. The molecular mass at 428.2965 (calcd for $C_{27}H_{40}O_4$, 428.2986, $[M]^+$) obtained from the HREIMS revealed a molecular formula of **54**. The NMR data (Table 14) of **54** were almost identical to those of **53**, however, with the absence of all acetate-related resonances. The ^{13}C NMR spectra exhibited two olefinic carbons at δ 127.4 (C-15) and 119.5 (C-16), corresponding to two protons at δ 6.43 (dd; J = 9.5, 3.0 Hz, H-16) and 5.68 (dd; J = 9.5, 3.0 Hz, H-15). Confirmed by the comparison with those reported by Kazlauskas *et al.* (1976), the structure of **54** was assigned as 16-deacetoxy-15,16-dehydroscalarafuran.



54

Table 14 ^1H and ^{13}C NMR data of compound 54 (C_6D_6; 500 MHz for ^1H)

positions	^1H (mult.; J in Hz)	^{13}C (mult.)
1 eq	1.53 (m)	39.9 (CH_2)
ax	0.61 (m)	
2 eq	1.40 (m)	18.1 (CH_2)
ax	1.24 (m)	
3 eq	1.49 (m)	42.3 (CH_2)
ax	1.31 (m)	
4	-	33.3 (C)
5	0.60 (dd; 12.5, 2.5)	56.5 (CH)
6 eq	1.38 (m)	18.9 (CH_2)
ax	0.65 (m)	
7 eq	1.70 (dt; 13.5, 3.5)	41.0 (CH_2)
ax	0.65 (m)	
8	-	36.9 (C)

Table 14 (cont.)

positions	^{1}H (mult.; *J* in Hz)	^{13}C (mult.)
9	0.61 (dd; 13.0, 2.0)	57.9 (CH)
10	-	37.5 (C)
11 eq	1.42 (m)	27.9 (CH_2)
ax	1.13 (m)	
12 ax	3.62 (br d; 8.5)	78.3 (CH)
13	-	40.9 (C)
14	1.81 (t; 3.3)	56.9 (CH)
15	5.68 (dd; 9.5, 3.0)	127.4 (CH)
16	6.43 (dd; 9.5, 3.0)	119.5 (CH)
17	-	121.5 (C)
18	-	133.4 (C)
19	7.71 (d; 1.5)	136.6 (CH)
20	7.09 (d; 1.5)	136.1 (CH)
21	0.85 (s; 3H)	33.5 (CH_3)
22	0.82 (s; 3H)	21.5 (CH_3)
23	0.74 (s; 3H)	16.3 (CH_3)
24	0.85 (s; 3H)	18.6 (CH_3)
25	1.08 (s; 3H)	16.3 (CH_3)

3.3 Biological activities of the scalarins

All the sesterterpenes obtained in this investigation were assessed for the antitubercular activity against *Mycobacterium tuberculosis* ($H_{37}Ra$) using MABA technique (Collins and Franzblau, 1997), and for cytotoxic activity against cancer cell line (MCF-7), using SRB assay (Skehan *et al.*, 1990). The results are shown in Table 15.

Table 15 Antitubercular activity and cytotoxicity of compounds **1**, **39** and **46**-**54**

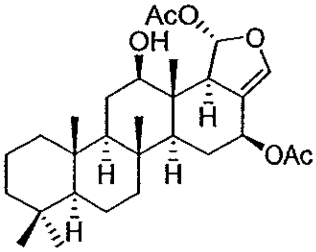
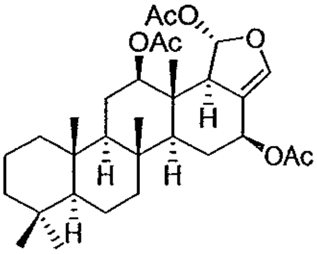
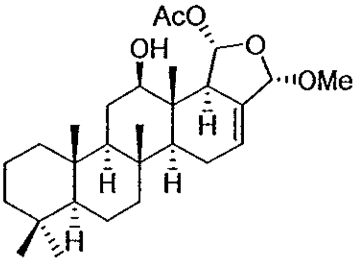
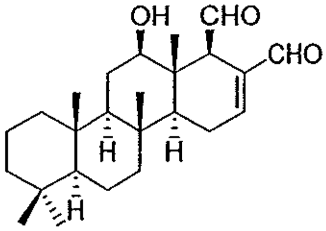
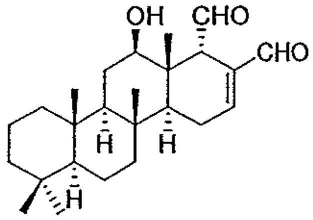
Compounds	Anti-TB (MIC; μM)	Cytotoxicity ($IC_{50} \pm SD$; nM)
 Heteronemin (1)	3	157 ± 13.55
 Heteronemin acetate (39)	3	612 ± 9.27
 12-*epi*-Deacetyl-19α-acetoxy-20α-methoxyscalaran (46)	54	315 ± 2.96
 12-Deacetyl-12-*epi*-scalaradial (47)	64	13 ± 0.77
 12-Deacetyl-12,18-di-*epi*-scalaradial (48)	Inactive[a]	122 ± 8.35

Table 15 (cont.)

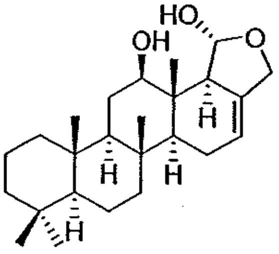
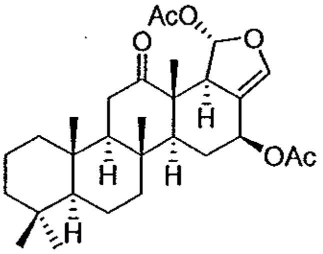
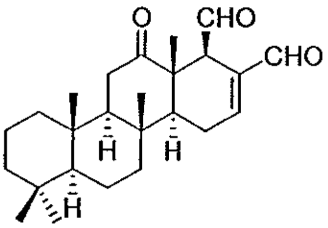
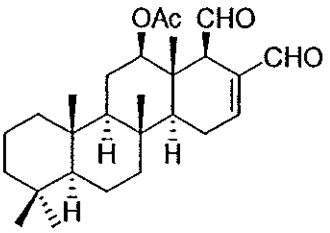
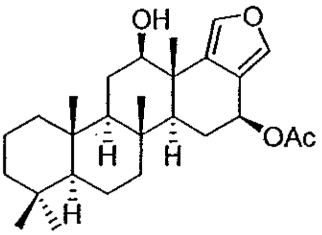
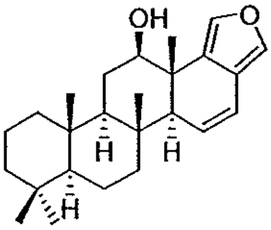
Compounds	Anti-TB (MIC; μM)	Cytotoxicity ($IC_{50} \pm SD$; nM)
 12-Deacetyl-12-*epi*-deoxoscalarin (49)	Inactive[a]	18 ± 5.73
 12-Oxoheteronemin (50)	0.23	17 ± 0.18
 12-Deacetyl-12-oxoscalaradial (51)	130	43 ± 5.04
 12-*epi*-Scalaradial (52)	58	474 ± 25.79
 Scalarafuran (53)	14	18 ± 0.35

Table 15 (cont.)

Compounds	Anti-TB (MIC; μM)	Cytotoxicity (IC$_{50}$ ± SD; nM)
 16-Deacetoxy-15,16-dehydro scalarafuran (54)	135	9 ± 1.12
Isoniazid	0.02	0.25 ± 0.02 mM
Rifampicin	0.04	1.5 ± 0.16 μM
Kanamycin	2.58	-
Camptothecin	-	0.72

Note; a) The tested sample were inactive at the highest concentration of 50 μg/mL.

The results demonstrated that all compounds, but **48** and **49**, exhibited antitubercular activity against *M. tuberculosis* with the MICs ranging from 0.23 to 135 μM. Among these, compounds **1**, **39** and **50** were the most potent with MICs in the magnitude of 10^{-1}-10^{0} μM. On the other hand, the significant cytotoxicity was observed with all targeted compounds, with the IC$_{50}$ in a nM range. Here, compound **54** was the most cytotoxicity (IC$_{50}$ 9 ± 1.12 nM). The antitubercular activities of **1** and **39** were all in good agreement with those previously reported (Wonganuchitmeta *et al.*, 2004). The antitubercular activity and cytotoxicity determination led to the conclusion regarding the structure-activity relationships of the scalarins as followed.

(I) The acetoxy group on C-16 was predominantly necessary for antitubercular activity. Compounds **1**, **39**, **50**, and **53**, each of which possessed the 16-acetoxy group, were strongly active, whereas compounds **46**, **47**, **51**, **52**, and **54** were found otherwise.

(II) The acetoxy group substituted as an acetal on C-19, in a subsequent manner to the 16-acetoxy group, also positively influenced the antitubercular activity. (See compounds **1**, **39**, **50**, and **53**.)

(III) The oxidation states and/or the aromaticity of the furan moiety did not post a strong effect on the antitubercular activity. However, this could be reflected from the fact that the effect from the furan moiety was overshadowed by those from the acetoxyl groups both on C-12 and C-19.

(IV) The polarity of the functionalities on C-12, therefore the electrostatic field on such region, affected the cytotoxicity on the MCF-7 cell line. The more polar of the functional groups, the stronger activity of the compounds. Thus, the 12-oxo derivatives were the least cytotoxic, whereas the hydroxyl ones were the most potent.

CHAPTER 4

CONCLUSION

Chemical investigation of the Thai sponge *Hyrtios* sp. from Koh-Tao, Surat Thani, led to the isolation of one new naturally-occurring sesterterpene, 12-*epi*-deacetyl-19α-acetoxy-20α-methoxyscalaran (46), along with a known sesterterpene, heteronemin (1). Using heteronemin as starting material, a series of chemically-derived scalarin derivatives were also obtained. These include heteronemin acetate (39), 12-deacetyl-12-*epi*-scalaradial (47), 12-deacetyl-12,18-di-*epi*-scalaradial (48), 12-deacetyl-12-*epi*-deoxoscalarin (49), 12-oxoheteronemin (50), 12-deacetyl-12-oxoscalaradial (51), 12-*epi*-scalaradial (52), scalarafuran (53) and 16-deacetoxy-15,16-dehydroscalarafuran (54).

The results from bioactivity evaluation of all the scalarins-type sesterterpenes (compounds 1, 39 and 46-54) showed the strong activities in both antitubercular and cytotoxic bioassays, with the MICs in antitubercular assay ranging from 0.23-135 μM and IC_{50}'s for cytotoxicity in a range of 9-612nM. Compounds 1, 39, and 47 were the most potent in antitubercular assay (MICs 3, 3 and 0.23 μM, respectively). On the other hand, compounds 47, 49, 50, 51, 53 and 54 were most cytotoxic (IC_{50}'s 13, 18, 17, 43, 18 and 9 nM, respectively). The incoherence between the antitubercular and cytotoxic activities suggested that the functionalities that affect each activity were different. Based on the chemical and activity profiles of the compounds investigated here, it can be proposed that the acetoxy functional groups on C-16 and C-19, in a subsequent manner, positively influence the antitubercular activity. For the cytotoxicity, the polarity of the oxygenated functionalities on C-12 affect the potency of the compounds.

Whereas certain conclusion regarding the structure-activity relationships can be drawn, it is too early to make an ultimate summary for the scalarins, as a series of extensive investigations are required. These include the in vitro cytotoxicity based on normal cell lines, selectivities of the compounds regarding other mycobacterial strains and cell lines, and larger library size that could represent the whole class of the compounds. All of these are now under investigation. Also, for drug design propose, a more mathematical conclusion on quantitative structure-activity relationship based on CoMFA and CoMSIA approaches is in progress.

Overall, this work has demonstrated that Thai marine organisms are among the potential sources of chemotherapeutic agents that may be useful in drug development. The further exploration will lead to the compounds with greater efficacy and specificity for the treatment of tuberculosis, as well as other infectious and cancerous diseases. The pharmaceutical potentials of marine bioresources hopefully will lead to the national policy for a better and more sustainable utilization of bioresources, as well as to a strong and positive impact on pharmaceutical research and development both in the governmental and public sectors in Thailand.

REFERENCES

Adams, L.L. and Luzzio, F.A. 1989. Ultrasound in oxochromium(VI)amine mediate oxidations-modification of the Corey-Suggs oxidation for the facile conversion of alcohols to carbonyl compounds. *J. Org. Chem.* 54: 5387-5390.

Allen, G. 1996. *Marine Life of Thailand and Southeast Asia.* Singapore: Periplus Editions Ltd.

Askin, D.; Angst, C. and Danishefsky, S. 1987. An approach to the synthesis of bactobolin and the total synthesis of *N*-acetyllactinobolamine: Some remarkably stable hemiacetals. *J. Org. Chem.* 52: 622-635.

Barron, P.F.; Quinn, R.J. and Tucker, D.J. 1991. Structure elucidation of a novel scalarane derivative by using high-field (14.1 T) NMR spectroscopy. *Aust. J. Chem.* 44: 995-999.

Barrow, C.J.; Blunt. J.W.; Munro, M.H.G. and Perry, N.B. 1988. Variabilin and related compounds from a sponge of the genus *Sarcotragus. J. Nat. Prod.* 51: 275-281.

Barsby, T.; Kelly, M.T. and Andersen, R.J. 2002. Tupuseleiamides and basiliskamides, new acyldipeptides and antifungal polyketides produced in culture by a *Bacillus laterosporus* isolate obtained from a tropical marine habitat. *J. Nat. Prod.* 65: 1447-1451.

Basak, A.; Nayak, M.K. and Chakraborti, A.K. 1998. Chemoselective *O*-methylation of phenols under nonaqueous condition. *Tetrahedron Lett.* 39: 4883-4886.

Bergquist, P.R. 1978. *Sponges.* Berkeley: University of Califonia Press.

Bourguet-Kondracki, M.L.; Martin, M.T.; Debitus, C. and Guyot, M. 1994. 12-*epi*-Heteronemin: new sesterterpene from the marine sponge *Hyrtios erecta. Tetrahedron Lett.* 35: 109-110.

Braekman, J.C.; Daloze, D.; Kaisin, M. and Moussiaux, B. 1985. Ichthyotoxic sesterterpenoids from the neo guinean sponge *Carteriospongia follascens. Tetrahedron.* 41: 4603-4614.

Cambie, R.C.; Rutledge, P.S.; Yang, X. and Bergquist, P.R. 1998. Chemistry of sponges. 18. 12-Deacetylfuroscalar-16-one, a new sesterterpene from a *Cacospongia* sp. *J. Nat. Prod.* 61: 1416-1417.

Cantrell, C.L.; Franzblau, S.G. and Fischer, N.H. 2001. Antimycobacterial plant terpenoids. *Planta Med.* 67: 685-694.

Chill, L.; Rudi, A.; Aknin, M.; Loya, S.; Hizi, A. and Kashman, Y. 2004. New sesterterpenes from Madacascan *Lendenfeldia* sponges. *Tetrahedron*. 60: 10619-10626.

Chung, G.A.C.; Aktar, Z.; Jackson, S. and Duncan, K. 1995. High-throughput screening for detecting antimycobacterial agents. *Antimicrob. Agents Chemocher.* 39: 2235-2238.

Cimino, G.; De Stefano, S. and Minale, L. 1973. Deoxoscalarin, a further sesquiterpene with the unusual tetracyclic carbon skeleton of scalarin, from the *Spongia officinalis*. *Experientia*. 30: 934-936.

Cimino, G.; De Stefano, S. and Minale, L. 1974. Scalaradial, a third sesquiterpene with the tetracarbocyclic skeleton of scalarin, from the *sponge Cacospongia mollior*. *Experientia*. 30: 846-847.

Cimino, G.; De Luca, P.; De Stefano, S. and Minale, L. 1975. Disidein, a pentacyclic sesterterpene condensed with an hydroxyhydroquinone moiety, from the sponge *Disidea pallascens*. *Tetrahedron*. 31: 271-275.

Cimino, G.; De Stefano, S.; Minale, L. and Trivellone, E. 1977. 12-*epi*-Scalarin and 12-*epi*-deoxoscalarin, sesterterpenes from the sponge *Spongia nitens*. *J. Chem. Soc., Perkin Trans.* 1: 1587-1593.

Cimino, G.; Cafieri, F.; De Napoli, L. and Fattorusso, F. 1978. ^{13}C-NMR spectrum and absolute stereochemistry of furoscalarol. *Tetrahedron Lett.* 23: 2041-2044.

Cimino, G.; De Stefano, S. and Di Luccia, A. 1979. Further sesterterpenes from the sponge *Spongia nitens*: 12-*epi*-scalaradial and 12,18-di-*epi*-scalaradial. *Experientia*. 35: 1277-1278.

Cimino, G.; De Rosa, S.; De Stefano, S.; Puliti, R.; Strazzullo, G.; Mattia, C.A. and Mazzarella, L. 1987. Absolute stereochemistry of disidein and of two new related halogenated sesterterpenoids. Two-dimentional NMR studies and X-ray crystal structure. *Tetrahedron*. 43: 4777-4784.

Collins, L.A. and Franzblau, S.G. 1997. Microplate alamar blue assay versus BACTEC 460 system for high-throughput screening of compounds against *Mycobacterium tuberculosis* and *Mycobacterium avium*. *Antimicrob. Agents Chemother.* 41: 1004-1009.

Cragg, G.M.; Newman, D.J. and Snader, K.M. 1996. Natural products in drug discovery and development. *J. Nat. Prod.* 60: 52-60.

Crews, P. and Bescansa, P. 1986. Sesterterpenes from a common marine sponge, *Hyrtios erecta*. *J. Nat. Prod.* 49: 1041-1052.

Crews, P.; Bescansa, P. and Bakus, G.P. 1985. A non-peroxide norsesterterpene from a marine sponge, *Hyrtios erecta*. *Experientia*. 41: 690-691.

Crews, P.; Jimenez, C.J. and Neil-Johnson, M.O. 1991. Using spectroscopic and database strategies to unravel structures of polycyclic bioactive marine sponge sesterterpenes. *Tetrahedron*. 47: 3585-3600.

Davis, R. and Capon, R.J. 1993. Two new scalarane sesterterpenes: Isoscalafuran-A and B, epimeric alcohol from a Southern Australian marine sponge, *Spongia hispida*. *Aust. J. Chem*. 46, 1295-1299.

De Freitas, J.C.; Blankmeier, L.A. and Jocobs, R.S. 1984. In vitro inactivation of the neurotoxic action of β-bungarotoxin by the marine natural product, manoalide. *Experientia*. 40: 864-865.

De Giulio, A.; De Rosa, S. and De Vincenzo, G. 1989. Terpenoids from the North Adriatic sponge *Spongia officinalis*. *J. Nat. Prod.* 52: 1258-1262.

De Rosa, S.; Puliti, R.; Crispino, A. and De Giulio, A. 1994. A new scalarane sesterterpenoid from the marine sponge *Cacospongia mollior*. *J. Nat. Prod.* 57: 256-262.

De Rosa, S.; Crispino, A.; De Giulio, A.; Iodice, C. and Tommonaro, G. 1998. A new dimethylscalarane derivative from the sponge *Cacospongia scalaris*. *Tetrahedron*. 54: 6185-6190.

Dewick, M. 1997. *Medicinal natural products, a biosynthetic approach*. Chichester: John Wiley & Sons Ltd.

Doi, Y.; Shigemori, H.; Ishibashi, M.; Mizobe, F.; Kawashima, A.; Nakaike, S. and Kobayashi, J. 1993. New sesterterpenes with nerve growth factor systhesis-stimulating activity from the Okinawan marine sponge *Hyrtios* sp. *Chem. Pharm. Bull.* 41: 2190-2191.

Donia, M. and Hamann, M.T. 2003. Marine natural products and their potential applications as anti-infective agents. *Lancet Infect. Dis.* 3: 338-348.

Duncan, K. 1997. Prospects for new anti-tuberculosis drugs. *J. Pharm. Pharmacol.* 49: 21-23.

Dye, C.; Scheele, S.; Dolin, P.; Pathania, V. and Rabiglione, M.C. 1999. Global burden of tuberculosis, estimated incidence, prevalence and mortality by country. *J. Am. Med. Assoc.* 282: 677-686.

Edrada, R.A.; Heubes, M.; Brauers, G.; Wray, V.; Berg, A.; Grafe, U.; Wohlfarth, M.; Muhlbacher, J.; Schaumann, K.; Sudarsono, S.; Bringmann, G. and Proksch, P. 2002. Online analysis of xestodecalactones A-C, novel bioactive metabolites from the fungus *Penicillium* cf. montanense and their subsequent isolation from the sponge *Xestospongia exigua*. *J. Nat. Prod.* 65: 1598-1604.

El Sayed, K.A.; Bartyzel, P.; Shen, X.; Perry, T.L.; Zjawiony, J.K. and Hamann, M.T. 2000. Marine natural products as antituberculosis agents. *Tetrahedron.* 56: 949-953.

Espinal, M.A. 2003. The globle situation of MDR-TB. *Tuberculosis.* 83: 44-51.

Fattorusso, E.; Magno, S.; Santacroce, C. and Sica, D. 1972. Scalarin, a new pentacyclic C-25 terpenoid from the sponge *Cacospongia scalaris*. *Tetrahedron.* 28: 5993-5997.

Faulkner, J. 1995. Chemical riches from the oceans. *Chemistry in Britain.* 31: 680-684.

Fontana, A.; Cavaliere, P.; Ungur, N.; D'Souza, L.; Parameswaram, P.S. and Cimino, G. 1999. New scalaranes from the nudibranch *Glossodoris atromarginata* and its sponge prey. *J. Nat. Prod.* 62: 1367-1370.

Fontana, A.; Mollo, E.; Ortea. J.; Gavagnin, M. and Cimino, G. 2000. Scalarane and homoscalarane compounds from the nudibranchs *Glossodoris sedna* and *Glossodoris dalli*: Chemical and biological properties. *J. Nat. Prod.* 63: 527-530.

Fu, X.; Zeng, L.M. and Su, J.Y. 1992. Scalarane-type bishomosesterterpenes from the sponge *Phyllospongia foliascens*. *J. Nat. Prod.* 55: 1607-1613.

Fu, X.; Zeng, L. and Su, J. 1993. Two new sesterterpenes from a South China sea sponge. *J. Nat. Prod.* 56: 1985-1988.

Fu, X.; Zeng, L.; Su, J. and Schmitz, F.J. 1999. Phyllofolactones C and D, two new minor homoscalarane sesterterpenes from the Chinese *sponge Phyllospongia foliascens*. *J. Nat. Prod.* 62: 644-646.

Gavagnin, M.; Mollo, E.; Docimo, T.; Guo, Y.W. and Cimino, G. 2004. Scalarane metabolites of the nudibranch *Glossodoris rufomarginata* and its dietary sponge from the South China sea. *J. Nat. Prod.* 67: 2104-2107.

Gerard, J.; Lloyd, R.; Barsby, T.; Haden, P.; Kelly, M.T. and Anderson, R.J. 1997. Massestolides A-H, antimycobacterial cyclic depsipeptides produced by two Pseudomanads isolated from marine habitats. *J. Nat. Prod.* 60: 223-229.

Glassroth, J. 2001. Tuberculosis, in *respiratory infectious*, pp. 475-486. Niederman *et al.*, eds. Philadelphia: Williams&Wilkins.

Hagiwara, H. and Uda, H. 1991. Total synthesis and absolute stereostructure of (+)-dysideapalaunic acid. *J. Chem. Soc., Perkin Trans. 1*: 1803-1807.

Hanson, J.R. 1992. The sesterterpenoids. *Nat. Prod. Rep.* 9: 481-489.

Hernández-Guerrero, J.C.; Zubía, E.; Ortiga, J.M. and Carballo, L.J. 2006. Sesterterpene metabolites from the sponge *Hyatella intestinalis*. *Tetrahedron*. 62: 5392-5400.

Hochlowski, J.E. and Faulkner, D.J. 1983. Metabolites of the dorid nudibranch *Chromodiris sedna*. *J. Org. Chem.* 48: 1733-1740.

Hu, J.F.; Schetz, J.A.; Kelly, M.; Peng, J.N.; Ang, K.K.; Flotow, H.; Leong, C.Y.; Ng, S.B.; Buss, A.D.; Wilkins, S.P. and Hamann, M.T. 2002. New antiinfective and human 5-HT2 receptor binding natural and semisynthetic compounds from the Jamaican sponge *Smenospongia aurea*. *J. Nat. Prod.* 65: 476-480.

Iguchi, K.; Saitoh, S. and Yamada, Y. 1989. Novel 19-oxygenated sterols from the Okinawan soft coral *Litophyton viridis*. *Chem. Pharm. Bull.* 37: 2553-2554.

Inderlied, C.B. 1999. Mycobacteria, in *infectious diseases*, pp. 1895-1916. Armstrong, D. and Cohen, J. eds. London: Harcourt publisher Ltd.

Jacob, R.S.; Culver, P.; Langdon, R.; Brien, T.O. and White, S. 1985. Some pharmacological observations on marine natural products. *Tetrahedron*. 41: 981-984.

Jiménez, J.I.; Yoshida, W.Y.; Scheuer, P.J. and Kelly, M. 2000. Scalarane-based sesterterpenes from an Indonesian sponge *Strepsichordaia aliena*. *J. Nat. Prod.* 63: 1388-1392.

Karuso, P.; Cambie, R.C. and Bowden, B.F. 1989. Chemistry of sponges. VI. Scalarane sesterterpenes from *Hyatella intestinalis*. *J. Nat. Prod.* 52: 89-293.

Kashman, Y. and Zviely, M. 1979. New alkylated scalarins from the *sponge Dysidea herbacea*. *Tetrahedron Lett.* 40: 3879-3882.

Kazlauskas, R.; Murply, P.T.; Quinn, R.J. and Wells, R.J. 1976. Heteronemin, a new scalarin type sesterterpene from the sponge *Heteronema erecta*. *Tetrahedron Lett.* 30: 2631-2634.

Kikuchi, H.; Tsukitani, Y.; Shimizu, I.; Kobayashi, M. and Kitagawa, I. 1983. Marine natural product. XI. An antiinflammatory scalarane-type bishomo-sesterterpene, foliaspongin, from the Okinawan marine sponge *Phyllospongia foliascens*. *Chem. Pharm. Bull.* 31: 552-556.

Kitagawa, I.; Kobayashi, M.; Lee, N.K.; Oyama, Y. and Kyogoky, Y. 1989. Marine natural products. XX. Bioactive scalarane-type bishomosesterterpenes from the Okinawan marine sponge *Phyllospongia foliascens*. *Chem. Pharm. Bull.* 37: 2078-2082.

Kittakoop, P.; Suttisri, R.; Chaichantipyuth, C.; Vethchagarun, S. and Suwanborirux, K. 1999. Norpregnane glycosides from a Thai soft coral, *Scleronephthya pallida*. *J. Nat. Prod.* 62: 318-320.

Kittakoop, P.; Wanasith, S.; Watts, P.; Kramyu, J.; Tanticharoen, M. and Thebtaranonth, Y. 2001. Potent antiviral potamogetonyde and potamogetonol, new furanoid labdane diterpenes from *Potamogeton malaianus*. *J. Nat. Prod.* 64: 385-388.

Kobayashi, M.; Okamoto, T.; Hayashi, K.; Yokoyama, N.; Sasaki, T. and Kitagawa, I. 1994. Marine natural products. XXXII. Absolute configurations of C-4 of the manoalide family, biologically active sesterterpenes from the marine sponge *Hyrtios erecta*. *Chem. Pharm. Bull.* 42: 265-270.

König, G.M. and Wright, A.D. 1996. Marine natural products research: current directions and future potential. *Planta Med.* 62: 193-211.

König, G.M.; Wright, A.D.; Sticher, O.; Angerhofer, C.K. and Pezzuto, J.M. 1994. Biological activities of selected marine natural products. *Planta Med.* 60: 532-537.

König, G.M.; Wright, A.D. and Franzblau, S.G. 2000. Assessment of anti- mycobacterial activity of a series of mainly marine derived natural products. *Planta Med.* 66: 337-342.

Kuehne, E.M.; Bornmann, G.W.; Parsons, H.W.; Spitzer, D.T.; Blount, F.J. and Zubietae, J. 1988. Total syntheses of (±)-cephalotaxine and (±)-8-oxocephalotaxine. *J. Org. Chem.* 53: 3439-3450.

Kupchan, S.M. and Tsou, G. 1973. Bruceantin, a new potent antileukemic simaroubolide from *Brucea antidysenterica*. *J. Org. Chem.* 38: 178-179.

Lan, W.J. and Li, H.J. 2007. New sesterterpenoids from the marine sponge *Phyllospongia papyracea*. *Helv. Chim. Acta.* 90: 1218-1222.

Li, H.J.; Amagata, T.; Tenney, K. and Crews, P. 2007. Additional scalarane sesterterpenes from the *Phyllospongia papyracea*. *J. Nat. Prod.* 70: 802-807.

Miyamoto, T.; Sakamoto, K.; Amano, H.; Arakawa, Y.; Nagarekawa, Y.; Komori, T.; Higuchi, R. and Sasaki, T. 1999. New cytotoxic sesterterpenoids from the nudibranch *Chromodoris inornata*. *Tetrahedron*. 55: 9133-9142.

Miyaoka, H.; Nishijima, S.; Mitome, H. and Yamada, Y. 2000. Three new scalarane sesterterpenoids from the Okinawan sponge *Hyrtios erectus*. *J. Nat. Prod.* 63: 1369-1372.

Molinsky, T.F. 1993. Marine pyridoacridine alkaloids: Structure, synthesis and biological chemistry. *Chem. Rev.* 93: 1825-1838.

Munro, M.H.G.; Blunt, J.W.; Dumdei, E.J.; Hickford, S.J.H.; Lill, R.E.; Li, S.; Battershill, C.N. and Duckworth, A.R. 1999. The Discovery and development of marine compounds with pharmaceutical potential. *J. Biotechnol.* 70: 15-25.

Nam, S.J.; Ko, H.; Shin, M.; Ham, J.; Kim, Y.; Kim, H.; Shin, K.; Choi, H. and Kang, H. 2006. Fanesoid X-activated receptor antanonists from a marine sponge *Spongia* sp. *Bioorg. Med. Chem. Lett.* 16: 5398-5402.

Nasu, S.S.; Yeung, B.K.S.; Hamann, M.T.; Scheuer, P.J.; Kelly-Borges, M. and Goins, K.D. 1995. Puupehenone-related metabolites from two Hawaiian sponges *Hyrtios* sp. *J. Org. Chem.* 60: 7290-7292.

N'Diaye, I.; Guella, G.; Mancini, I.; Kornprobst, J. and Pietra, F. 1991. Konakhin, a novel type of degraded sesterterpene; Isolation from a marine sponge of Senegal. *J. Chem. Soc., Chem. Commun.* 97-98.

Newton, S.M.; Lau, C. and Wright, C.W. 2000. A review of antimycobacterial natural products. *Phytotherapy Resea.* 14: 303-322.

Nicholas, G.M.; Eckman, L.L.; Newton, G.L.; Fahey, R.C.; Ray, S. and Bewley, C.A. 2003. Inhibition and kinetics of *mycobacterium tuberculosis* and *mycobacterium smegmatis* mycothiol-S-conjugate amidase by natural product inhibitors. *Bioorg. Med. Chem.* 11: 601-608.

Pettit, G.R.; Cichacz, Z.A.; Tan, R.; Hoard, M.S.; Melody, N. and Pettit, R.K. 1998. Isolation of sesterstatins 1-3 from the marine sponge *Hyrtios erecta*. *J. Nat. Prod.* 61: 13-16.

Pettit, G.R.; Tan, R. and Cichacz, Z.A. 2005. Antineoplastic agents. 524. Isolation and structure of sesterstatin 6 from the Indian ocean sponge *Hyrtios erecta*. *J. Nat. Prod.* 68: 1235-1255.

Phuwapraisirisan, P.; Matsunaga, S.; Fusetani, N.; Chaitanawisuti, N.; Kritsanapuntu, S. and Menasveta, P. 2003. Mycaperoxide H, a new cytotoxic nor-sesterterpene peroxide from a Thai marine sponge *Mycale* sp. *J. Nat. Prod.* 66: 289-291.

Ponomarenko, P.L.; Kalinnovsky, I.A. and Stonik, A.V. 2004. New scalarane-based sesterterpenes from the sponge *Phyllospongia madagascarensis*. *J. Nat. Prod.* 67: 1507-1510.

Qiu, Y.; Deng, Z.; Pei, Y.; Fu, H.; Proksch, P. and Lin, W. 2004. Sesterterpenoids from the marine sponge *Hyrtios erectus*. *J. Nat. Prod.* 67: 921-924.

Rodriguez, A.D. and Ramirez, C. 2001. Serrulatane diterpenes with antimycobacterial activity isolated from the West Indian sea whip *Pseudopterogorgia elisabethae*. *J. Nat. Prod.* 64: 100-102.

Rodriguez, A.D.; Ramirez, C.; Rodriguez, I.I. and Gonzalez, E. 1999. Novel anti-mycobacterial benzoxazole alkaloids, from the West Indian sea whip *Pseudopterogorgia elisabethae*. *Org. Lett.* 1: 527-530.

Rodriguez, I.I. and Rodrguez, A.D. 2003. Homopseudopteroxazole, a new antimycobacterial diterpene alkaloid from *Pseudopterogorgia elisabethae*. *J. Nat. Prod.* 66: 855-857.

Rueda, A.; Zubia, E.; Ortega, M.J.; Carballo, J.L. and Salva, J. 1997. New cytotoxic metabolites from the sponge *Cacospongia scalaris*. *J. Org. Chem.* 62: 1481-1485.

Ryu, G.; Matusunaga, S. and Fusetani, N. 1996. Three new cytotoxic sesterterpenes from the marine sponge *Hyrtios* cf. *erectus*. *J. Nat. Prod.* 59: 515-517.

Sakai, R. and Higa, T. 1986. Manzamine A, a novel antitumor alkaloid from a sponge. *J. Am. Chem. Soc.* 108: 6404-6405.

Scheuer, P.J. 1990. Some marine ecological phemonema: Chemical basis and biomedical potential. *Science.* 248: 173-177.

Sensi, P. and Grassi, G.G. 1996. Antimycobacterial agents, *in burger's medicinal chemistry and drug discovery: therapeutic agents.* 2: 575-635, Wolff *et al.*, eds. New York: John wiley&Sons, Inc.

Shi, Y.P.; Rodrguez, A.D.; Barnes, C.L.; Sanchez, J.A.; Raptis, R.G. and Baran, P. 2002. New terpenoid constituents from *Eunicea pinta*. *J. Nat. Prod.* 65: 1232-1241.

Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J.T.; Bokesch, H.; Kenney, S. and Boyd, M.R. 1990. New colorimetric cytotoxic assay for anticancer-drug screening. *J. Natl. Cancer Inst.* 82: 1108-1112.

Tanaka, J.; Higa, T.; Suwanborirux, K.; Kokpol, U.; Bernardinelli, G. and Jefford, C.W. 1993. Bioactive norsesterterpene 1,2-dioxanes from a Thai sponge, *Mycale* sp. *J. Org. Chem.* 58: 2999-3002.

Terem, B. and Scheure, P.J. 1986. Scalaradial derivatives from the nudibranch *Chromodoris youngbleuthi* and the sponge *Spongia oceania*. *Tetrahedron*. 42: 4409-4412.

Tokou, T.; Miura, S.; Kato, H.; Hirota, H.; Ohta, T. and Tsukamoto, S. 2006. Neurotrophic sesterterpenes isolated from a marine sponge, *Spongia* sp. *Heterocycles*. 69: 521-526.

Walker, R.P.; Thompson, J.E. and Faulkner, D.J. 1980. Sesterterpenes from *Spongia idia*. *J. Org. Chem.* 45: 4976-4979.

Watanadilok, R.; Sonchaeng, P.; Kijjoa, A.; Damas, A.M. and Gales, L. 2000. Tetillapyrone and nortetillapyrone, two unusual hydroxypyran-2-ones from the marine sponge *Tetilla japonica*. *J. Nat. Prod.* 64: 1056-1058.

Wonganuchitmeta S.; Yuenyongsawad, S.; Keawpradub, N. and Plubrukarn, A. 2004. Antitubercular sesterterpenes from the Thai sponge *Brachiaster* sp. *J. Nat. Prod.* 67: 1767-1770.

World Health Organization. 2007. Global tuberculosis control: Surveillance, planning, financing, *WHO report 2007*. Geneva, Switzerland.

Yasuda, F. and Tada, H. 1981. Desacetylscalaradial, a cytotoxic metabolite from the sponge *Cacospongia scalaris*. *Experientia*. 37: 110-111.

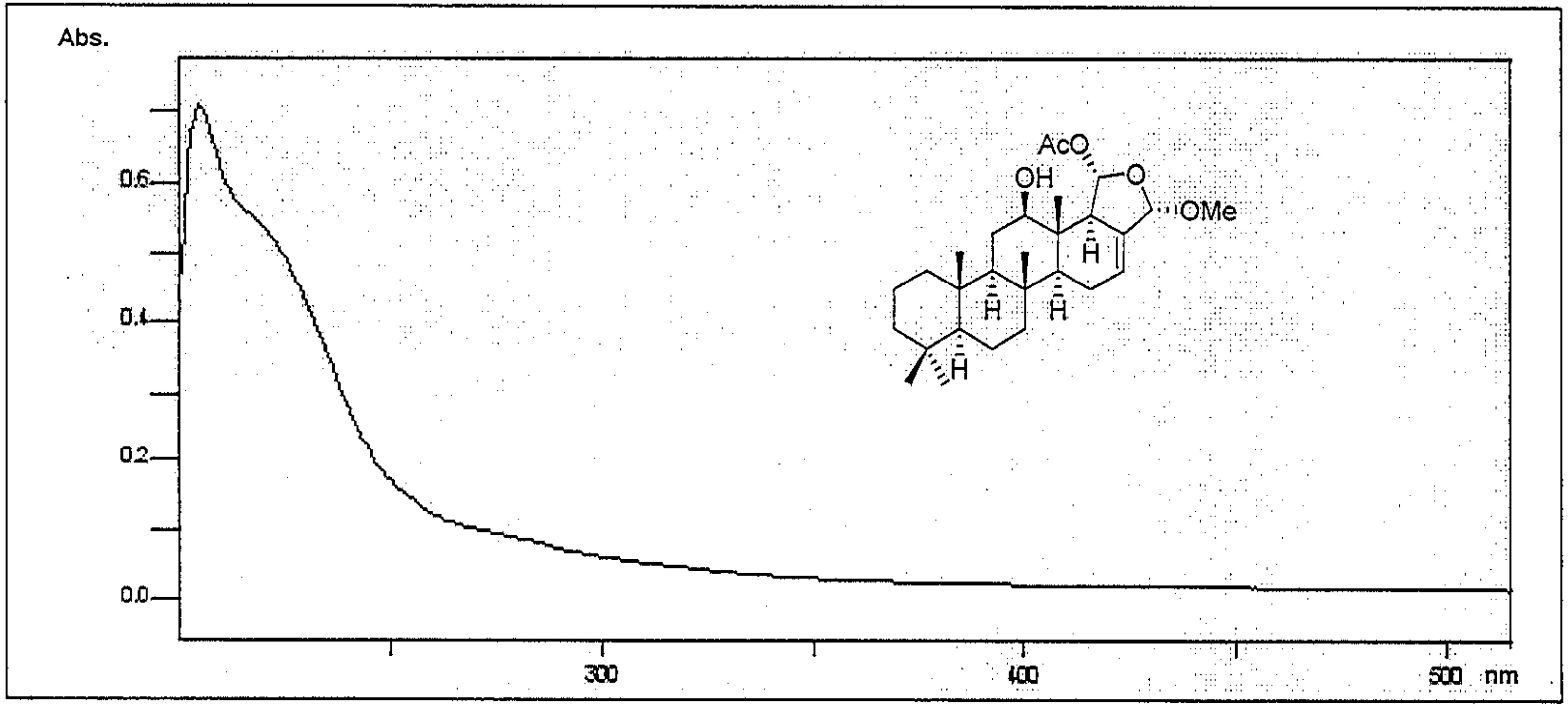
Youssef, D.T.A.; Yamaki, R.K.; Kelly, M. and Scheuer, P.J. 2002. Salmahyrtisol A, a novel cytotoxic sesterterpene from the red sea sponge *Hyrtios erecta*. *J. Nat. Prod.* 65: 2-6.

Youssef, D.T.A.; Shaala, L.A. and Emara, S. 2005. Antimycobacterial scalarane-based sesterterpenes from the red sea sponge *Hyrtios erecta*. *J. Nat. Prod.* 68: 1782-1784.

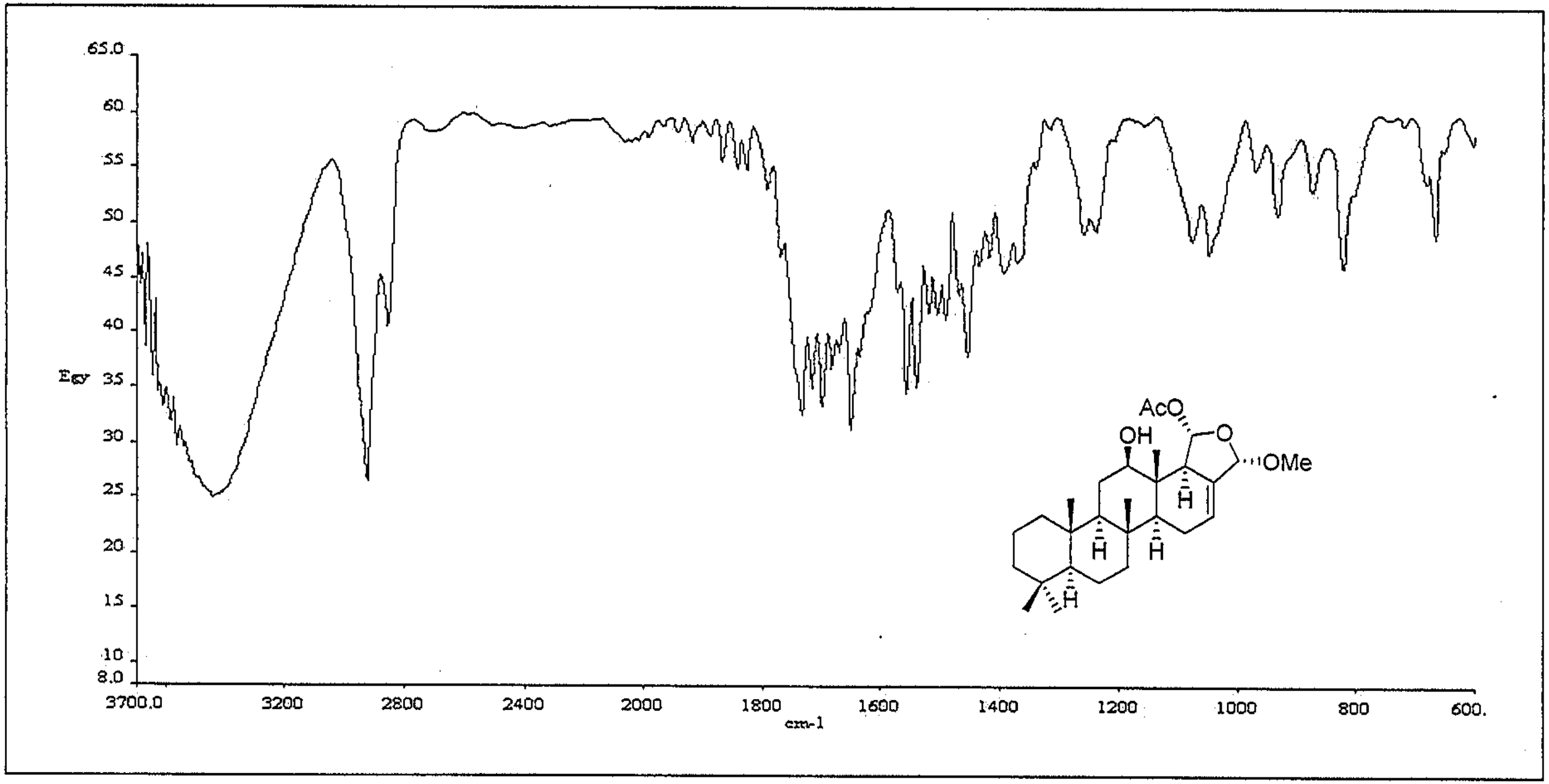
Yu, Z.G.; Bi, K.S. and Guo, Y.W. 2005. Hyrtiosins A-E, five new scalarane sesterterpenes from the South Chaina sea sponge *Hyrtios erecta*. *Helv. Chim. Acta.* 88: 1004-1009.

Zeng, L.; Fu, X. and Su, J. 1991. Novel bishomoscalarane sesterterpenes from the sponge *Phyllospongia foliascens*. *J. Nat. Prod.* 54: 421-427.

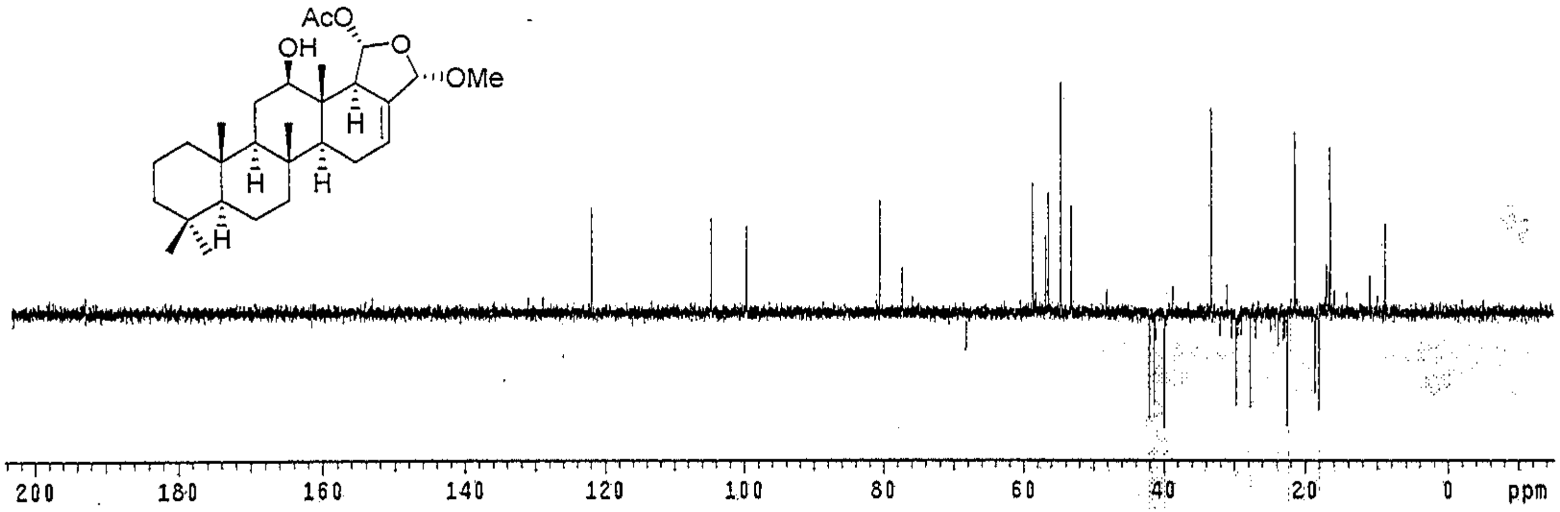
APPENDIX



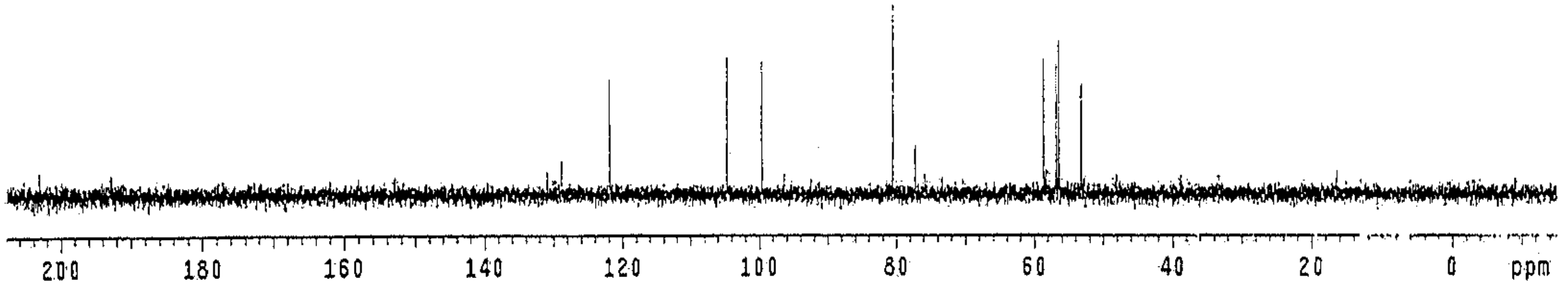
UV spectrum of compound 46 (MeOH)



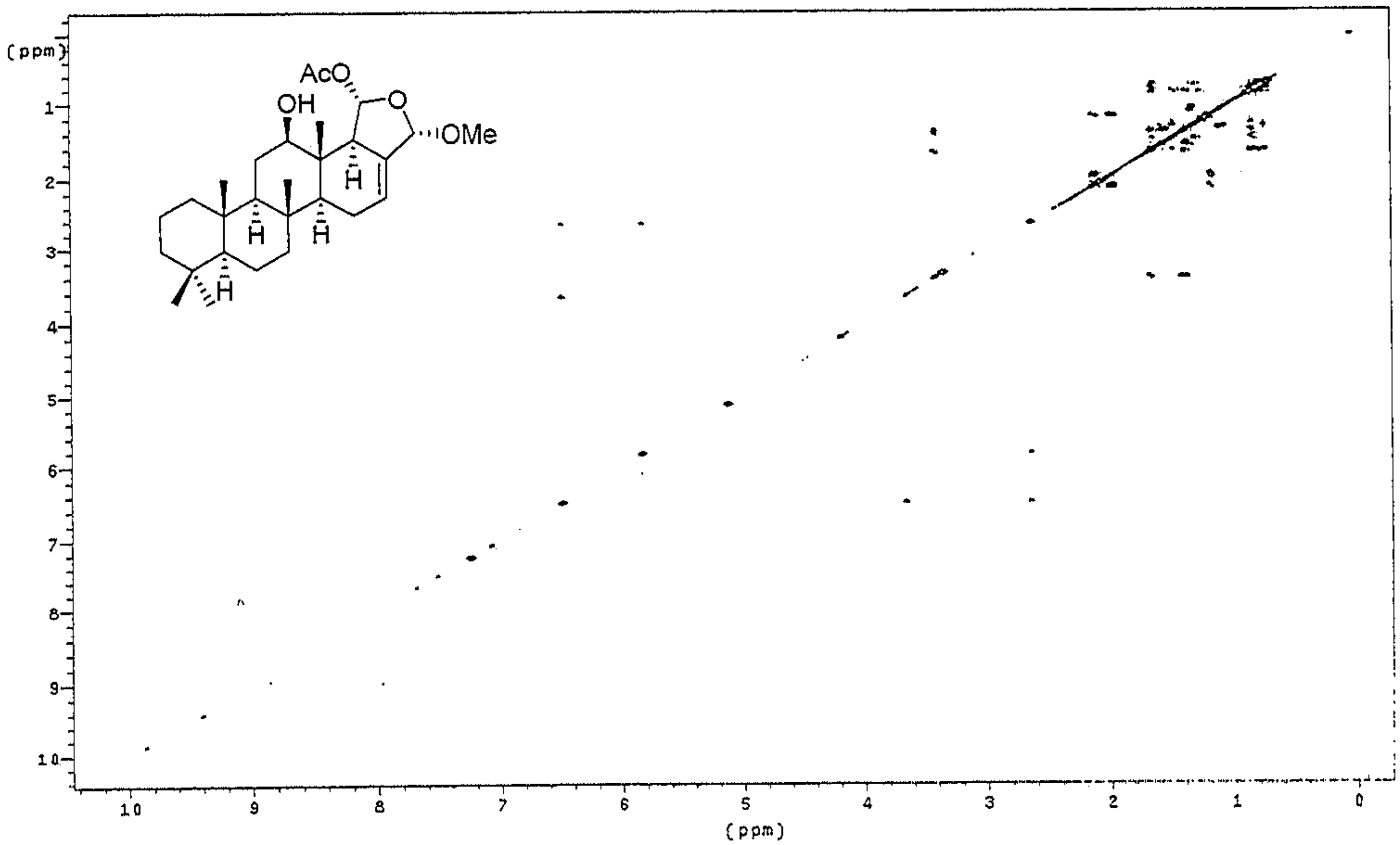
IR spectrum of compound 46 (neat)



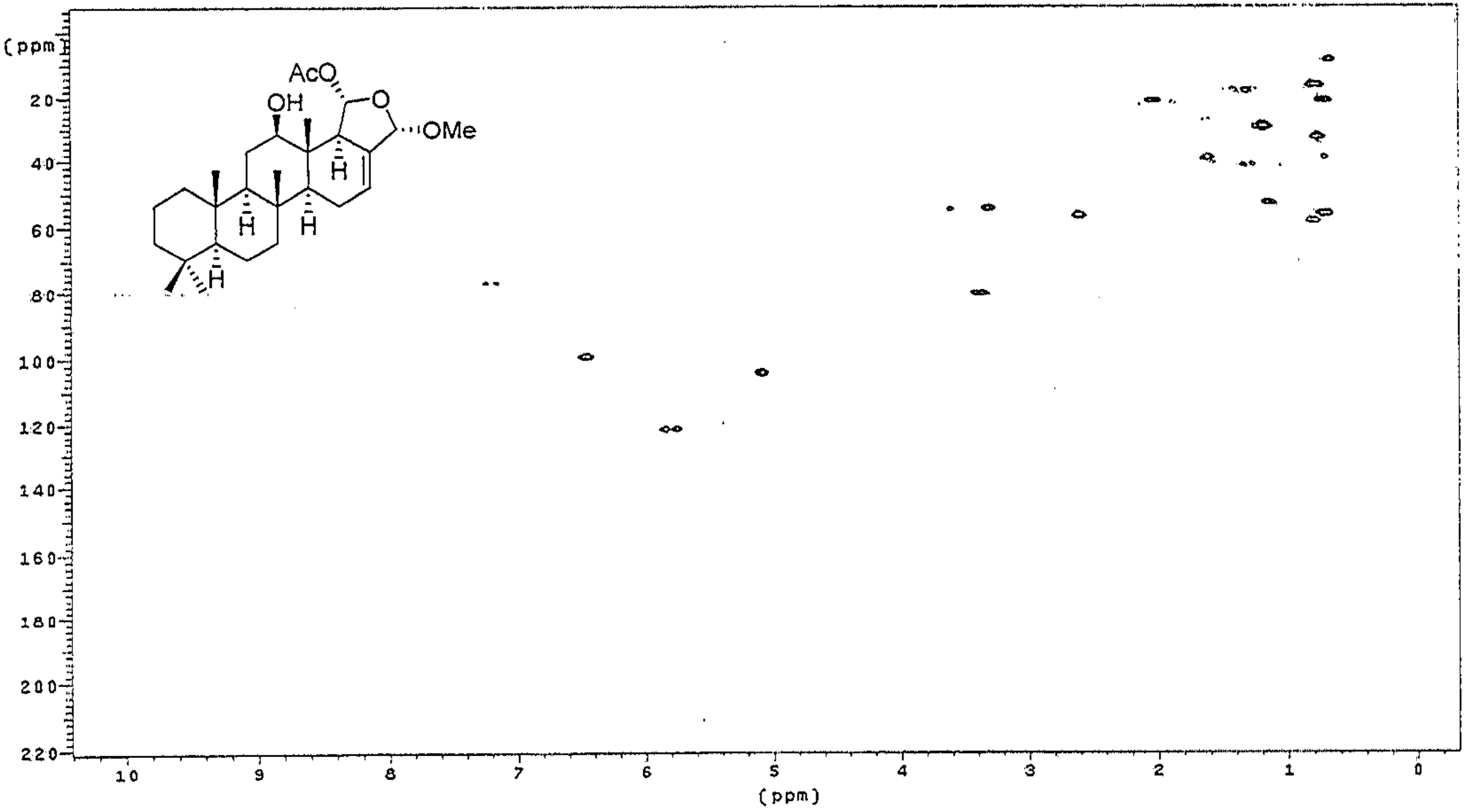
DEPT 135° spectrum of compound **46** ($CDCl_3$)



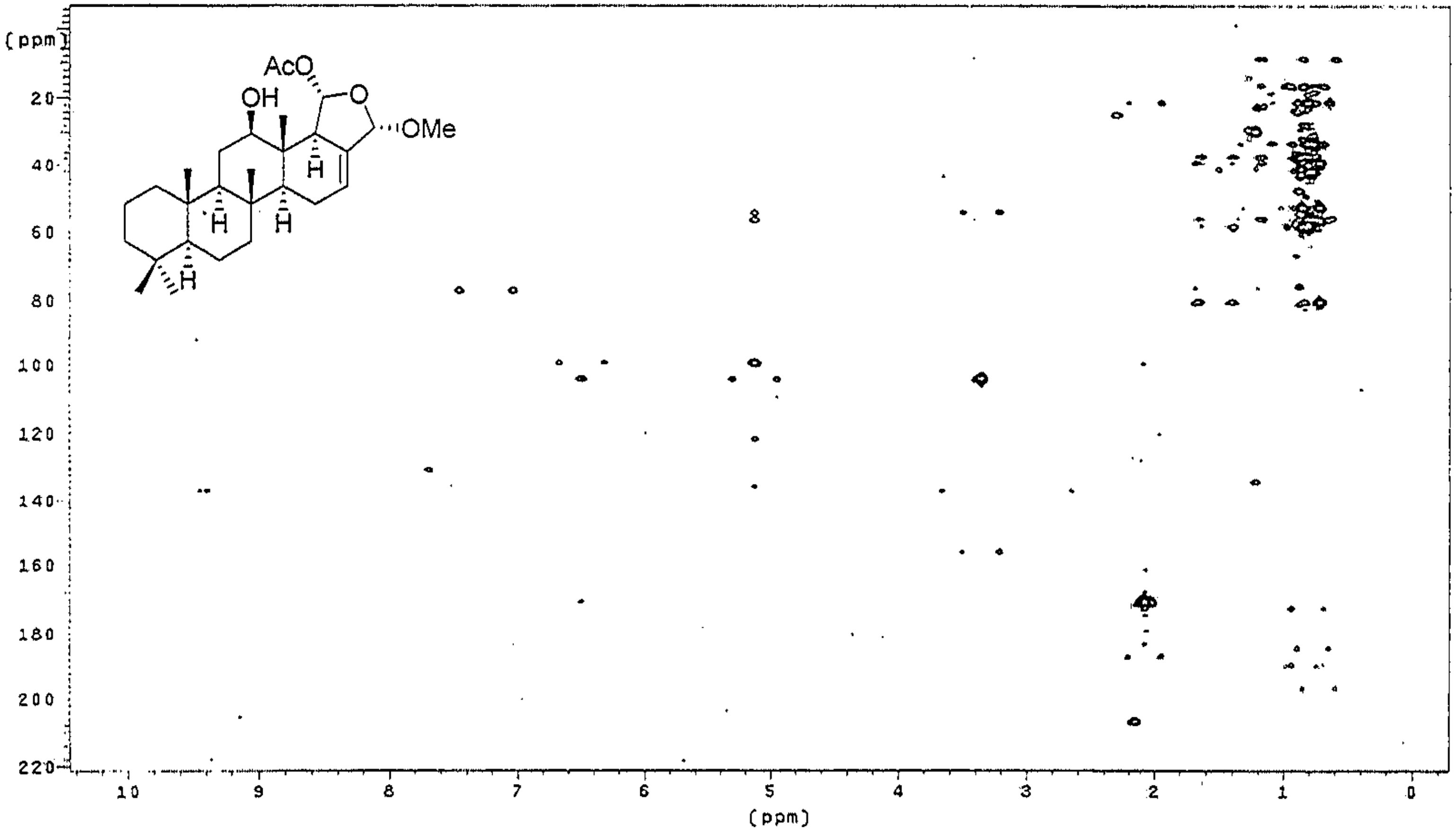
DEPT 90° spectrum of compound **46** ($CDCl_3$)



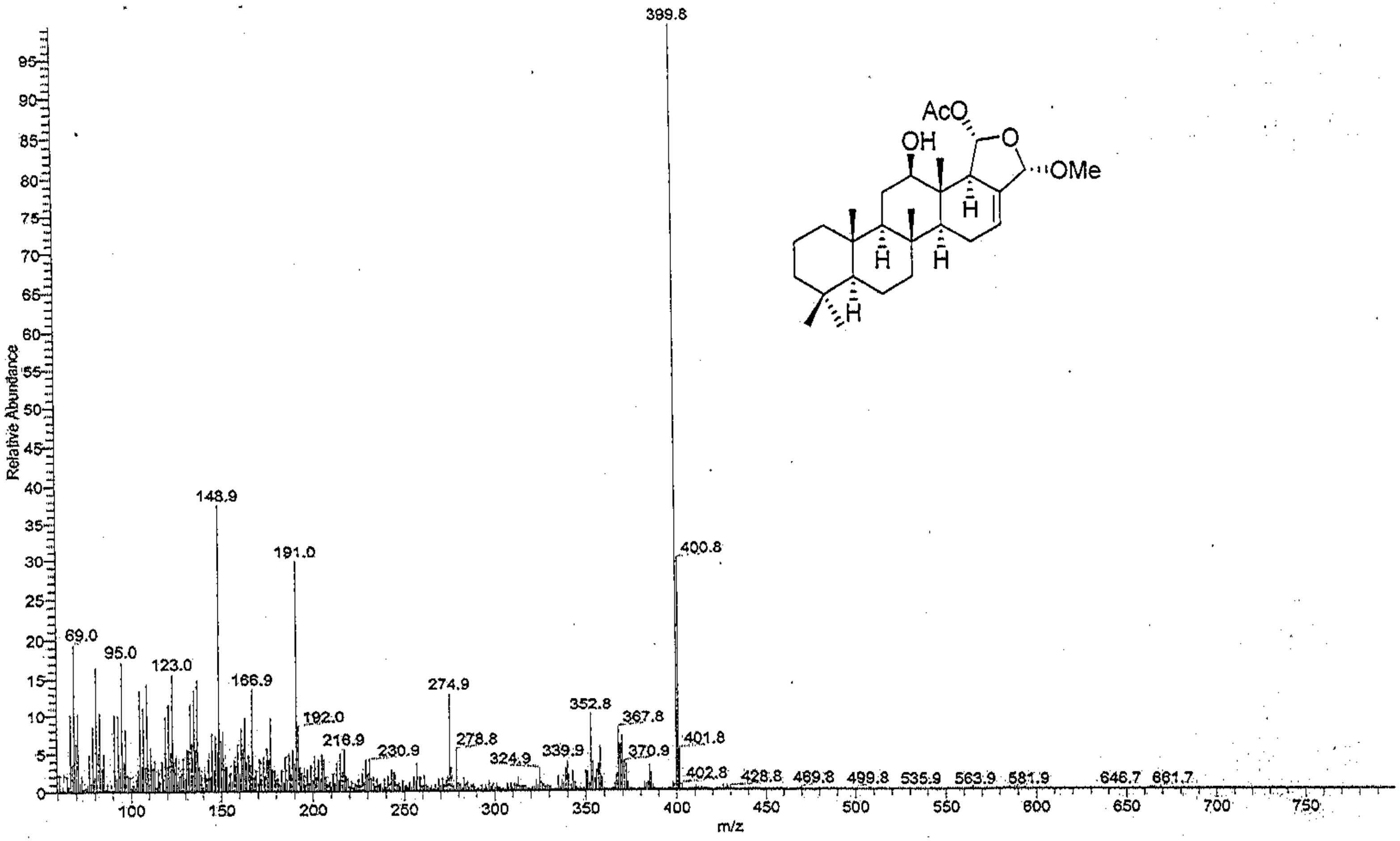
^{1}H-^{1}H COSY spectrum of compound 46 (CDCl$_3$)



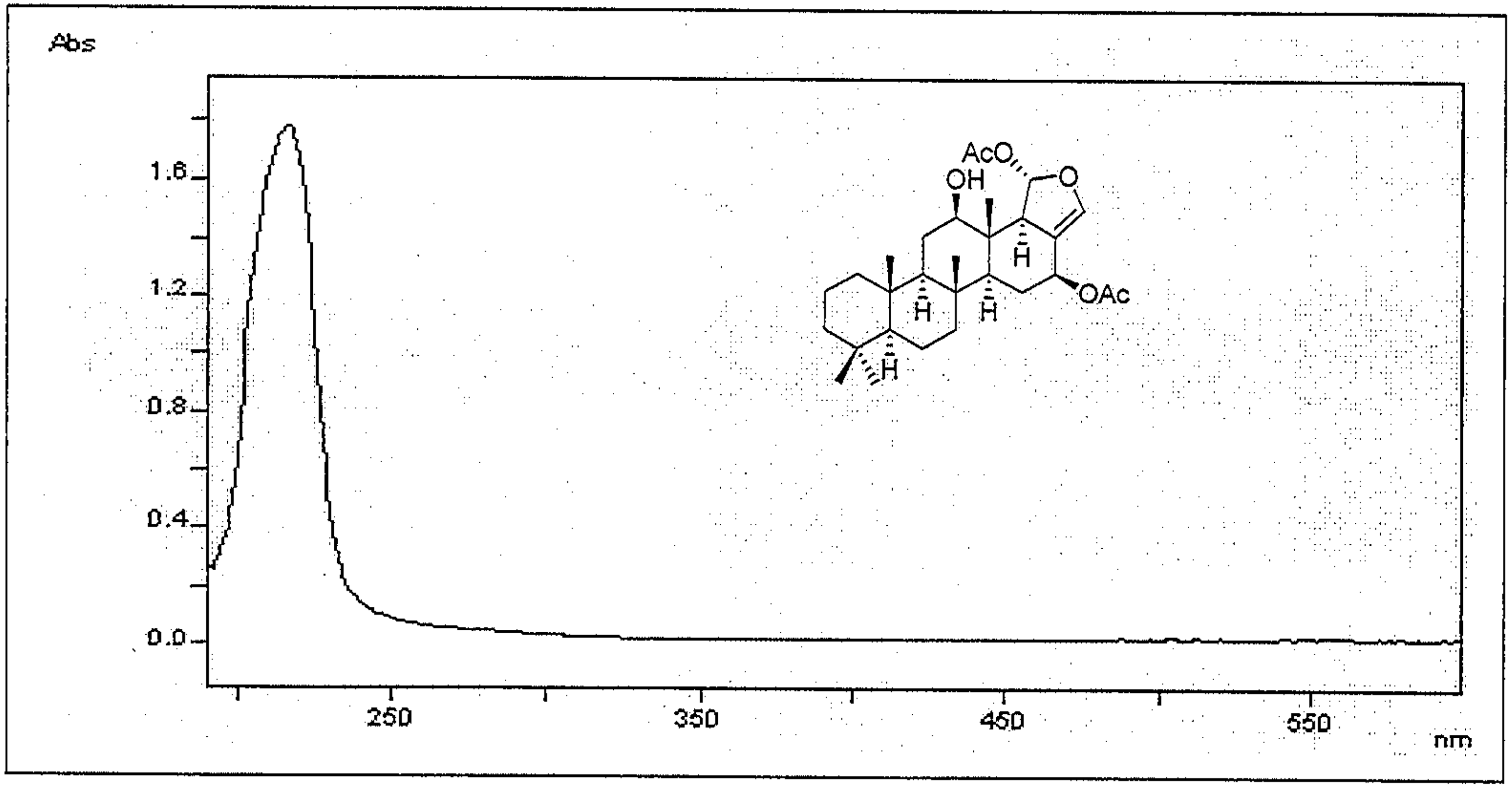
HMQC spectrum of compound 46 ($CDCl_3$)



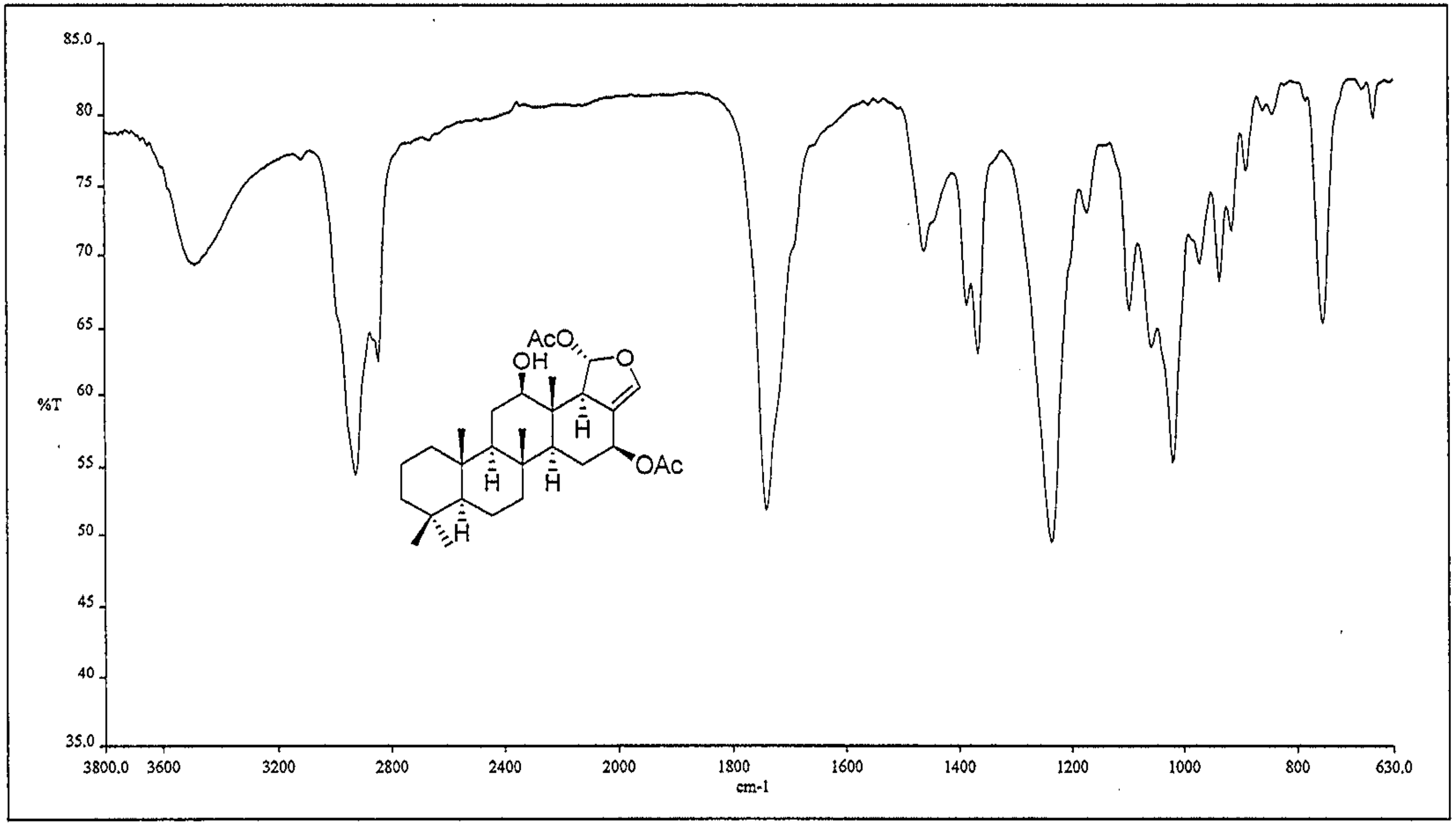
HMBC (C_6D_6) spectrum of compound 46 ($CDCl_3$)



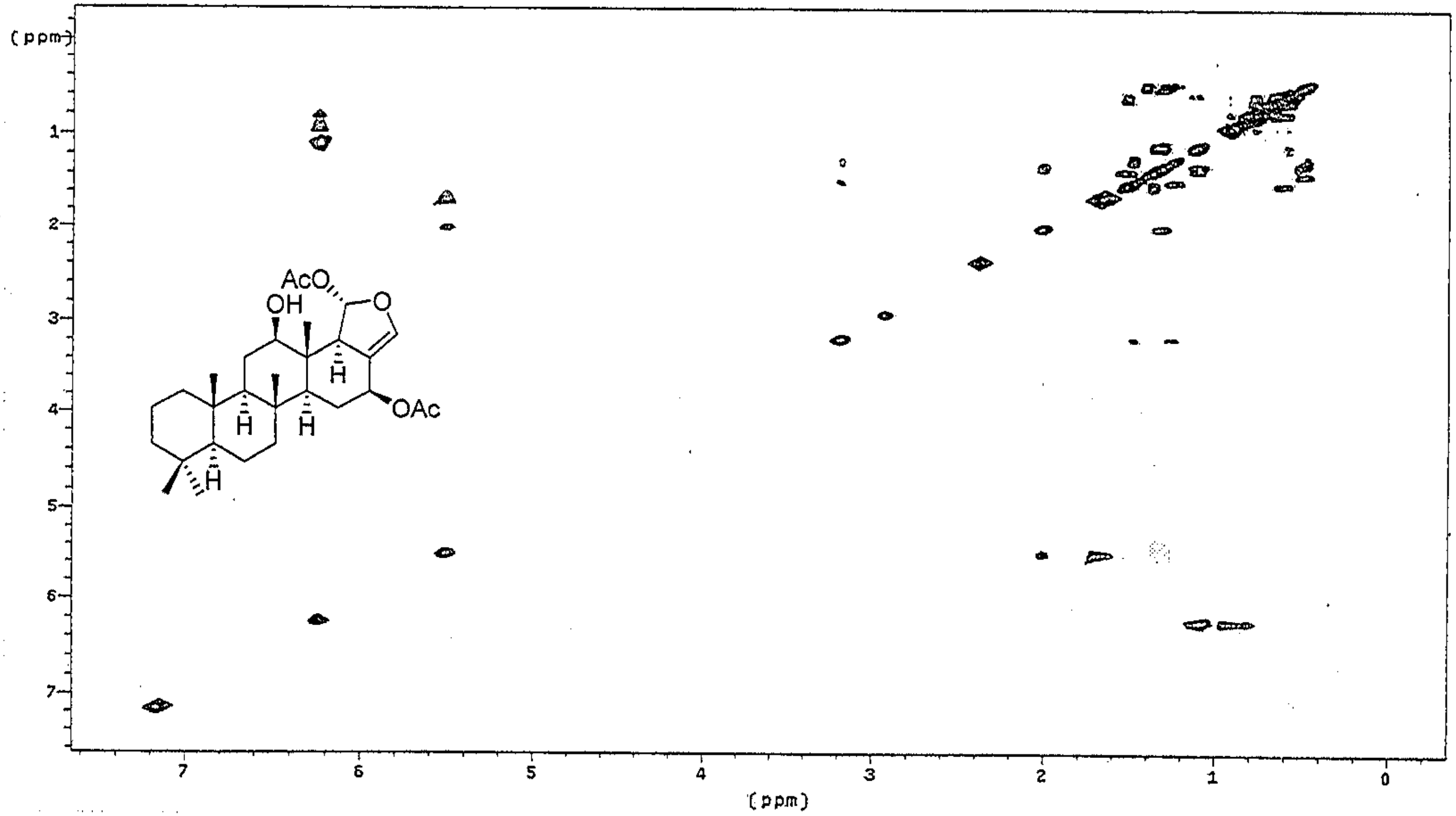
EI mass spectrum of compound 46



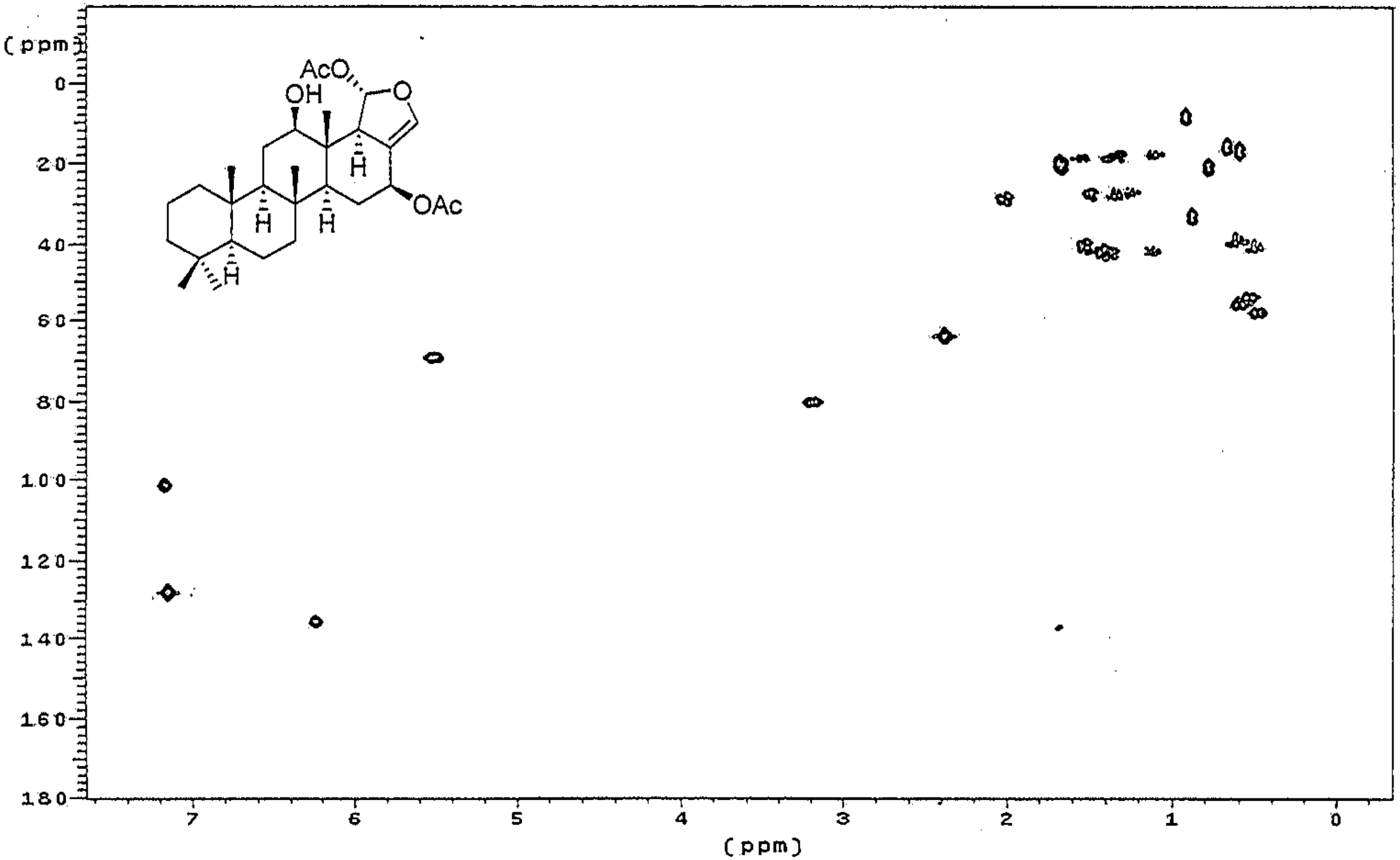
UV spectrum of compound **1** (MeOH)



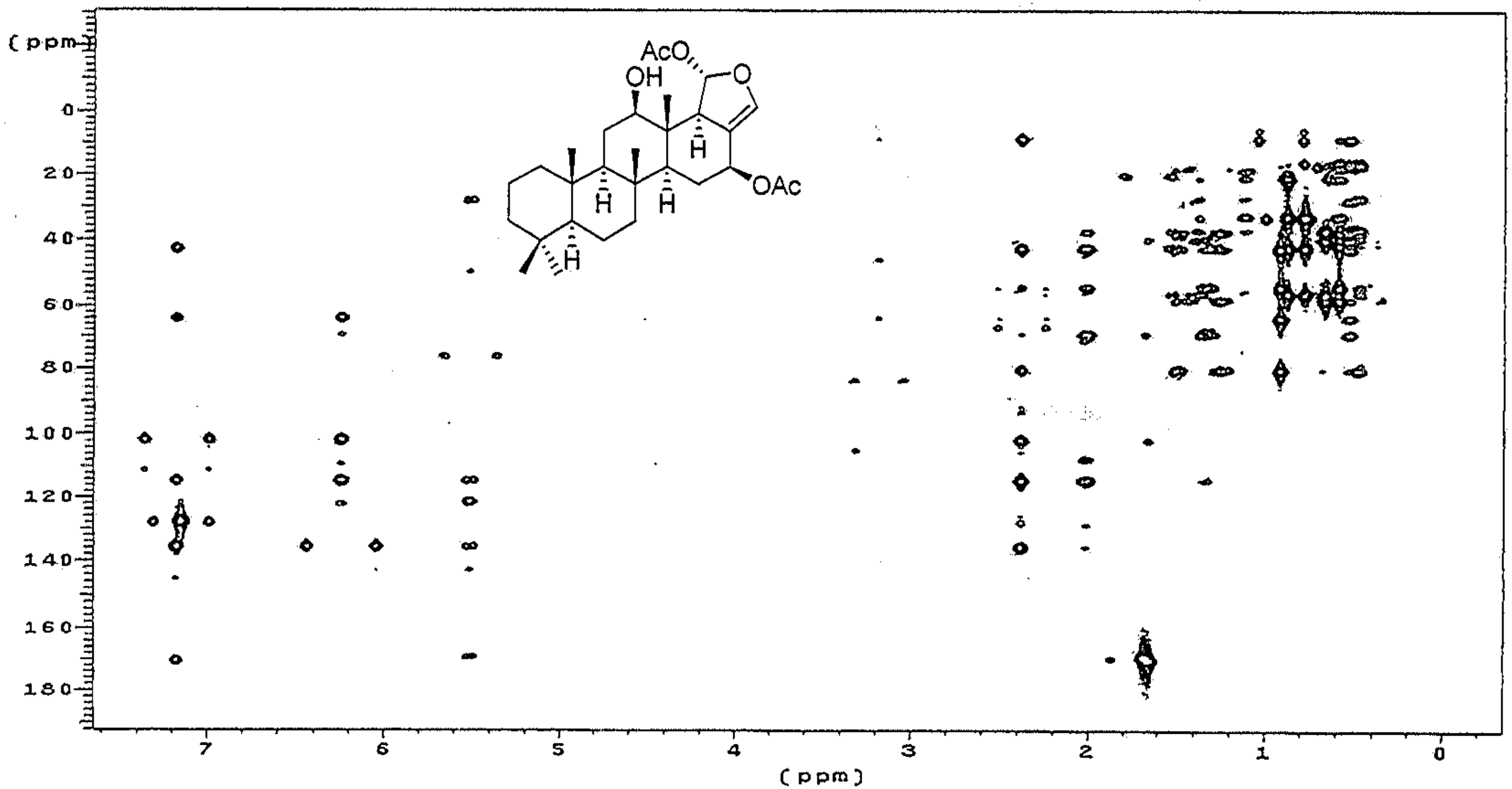
IR spectrum of compound 1 (neat)



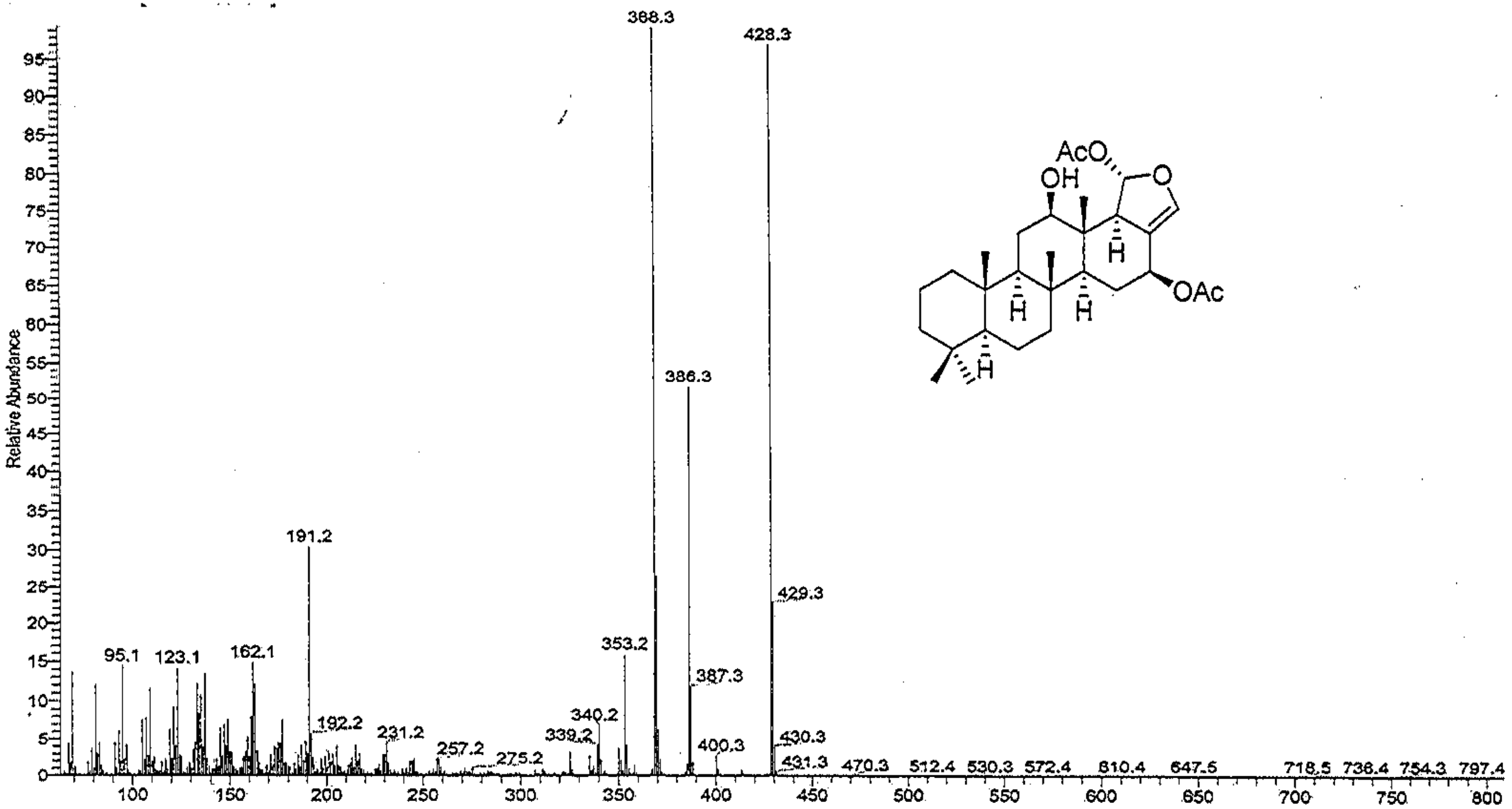
^{1}H-^{1}H COSY spectrum of compound **1** (C_6D_6)



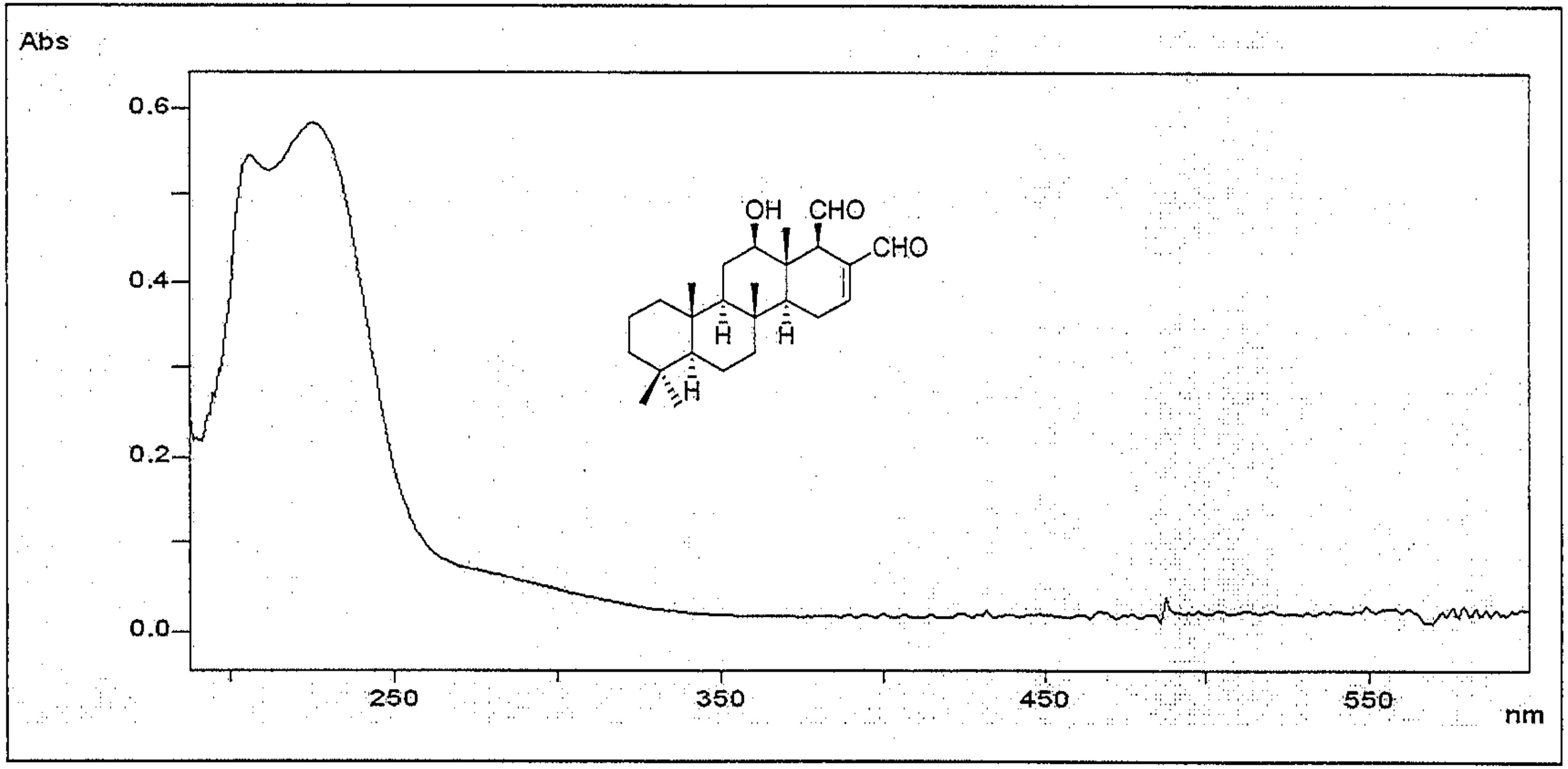
HMQC spectrum of compound **1** (C$_6$D$_6$)



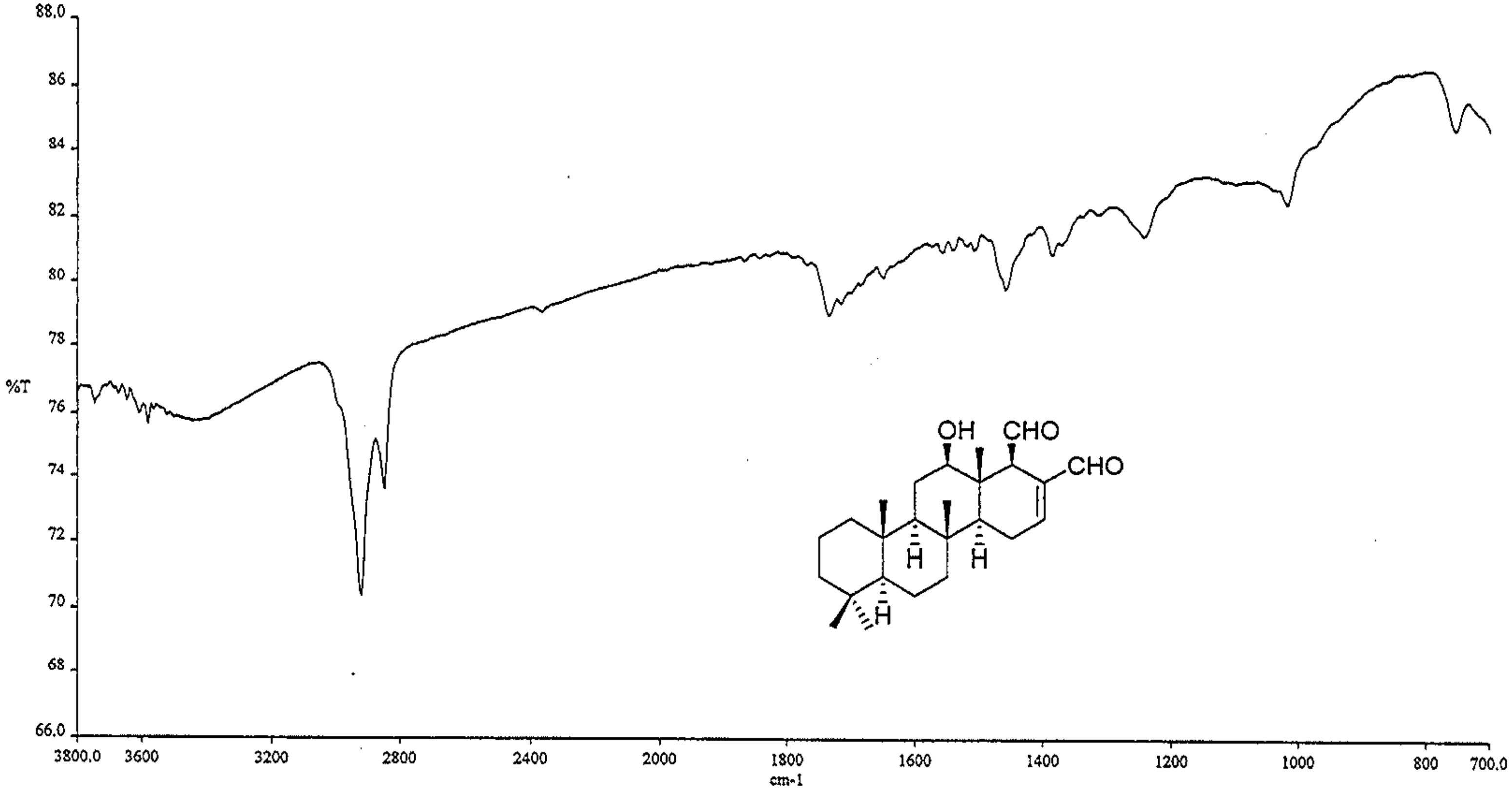
HMBC spectrum of compound **1** (C_6D_6)



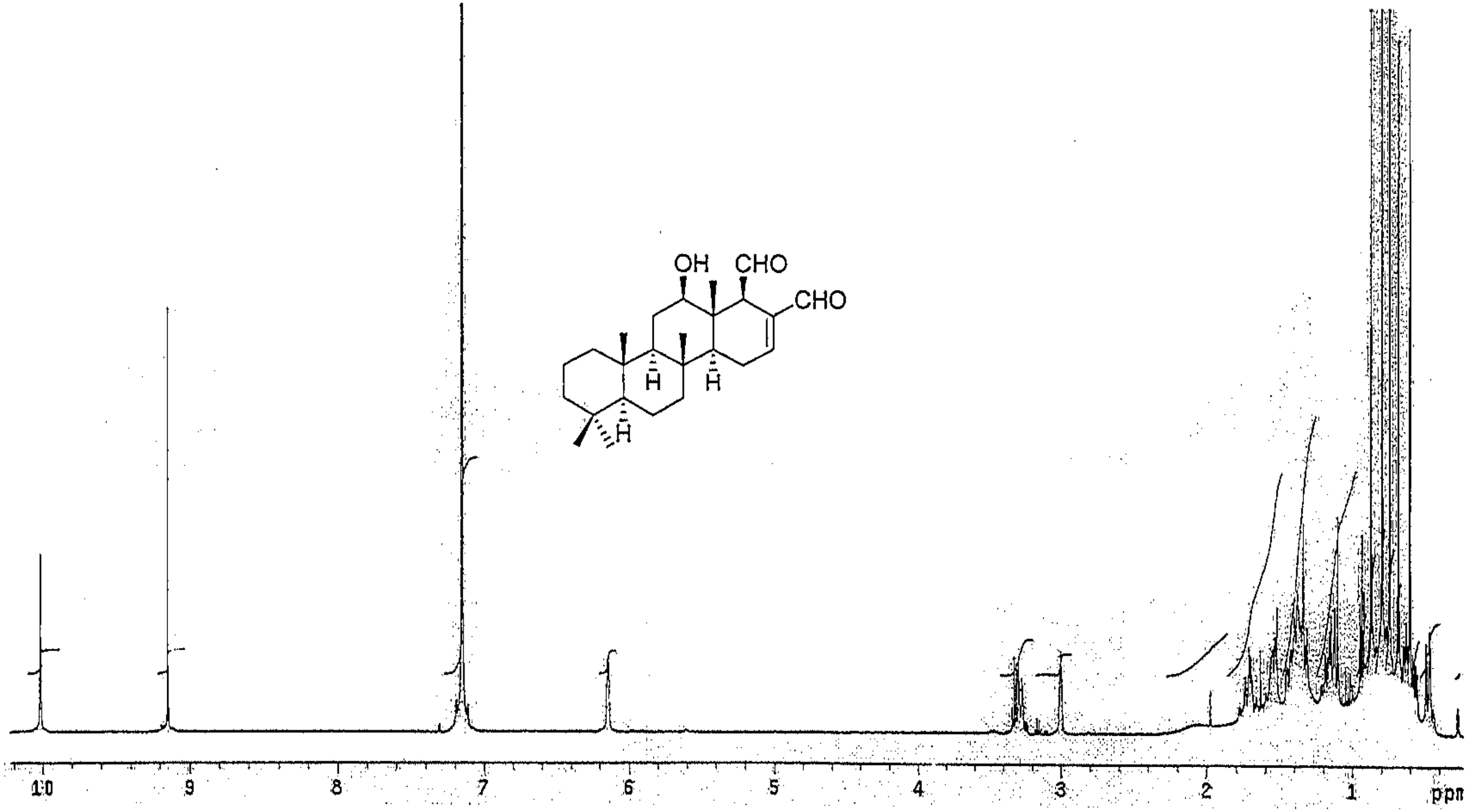
EI mass spectrum of compound 1



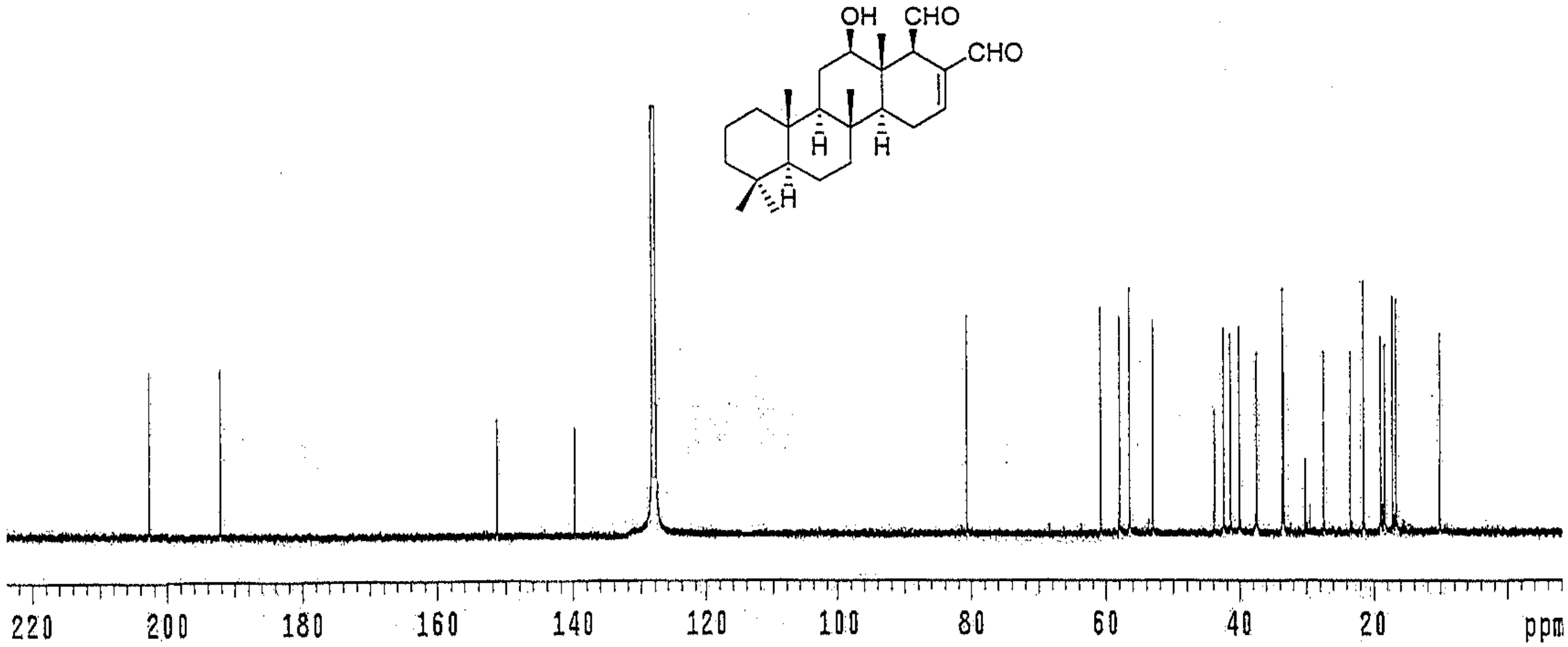
UV spectrum of compound **47** (MeOH)



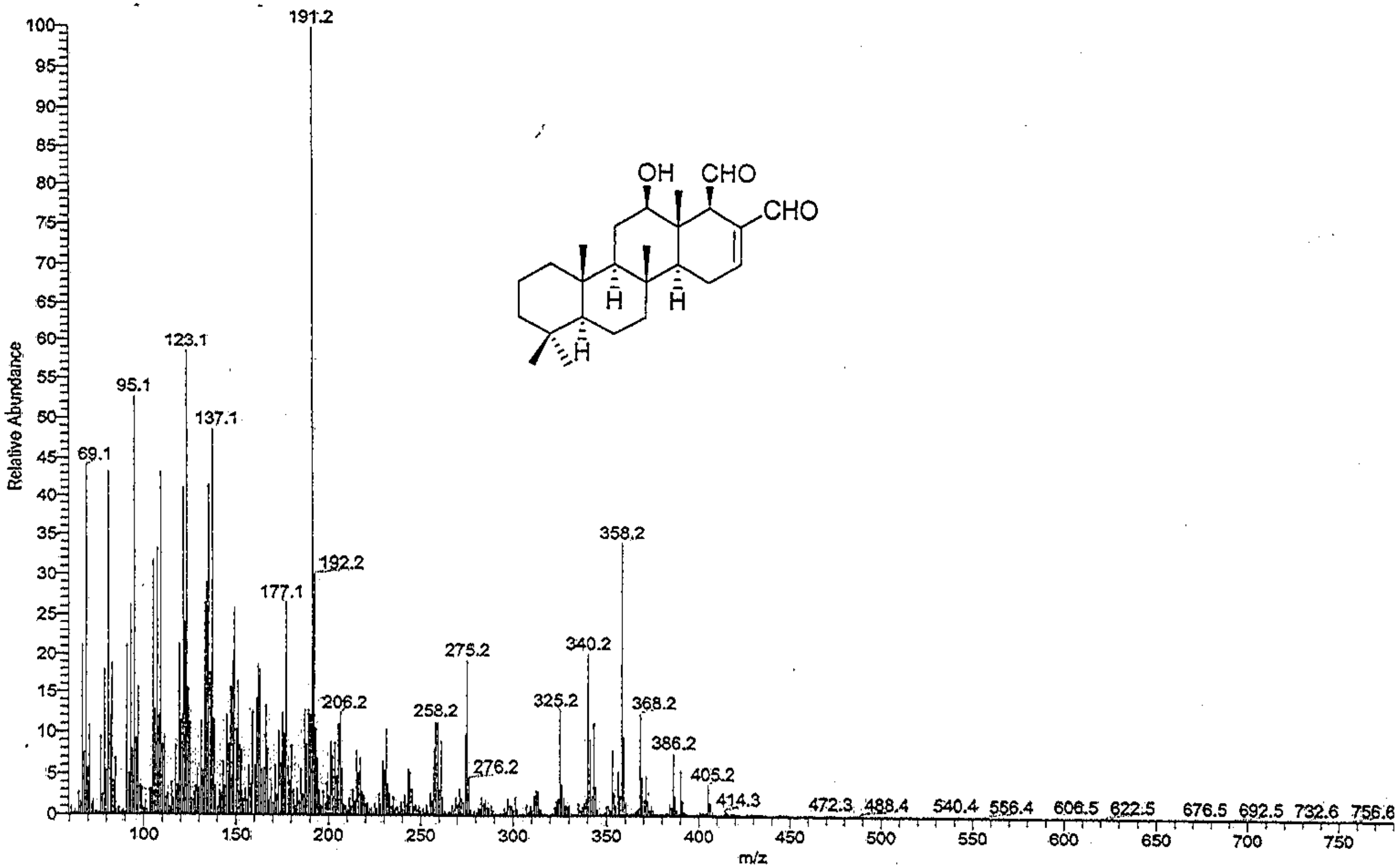
IR spectrum of compound **47** (neat)



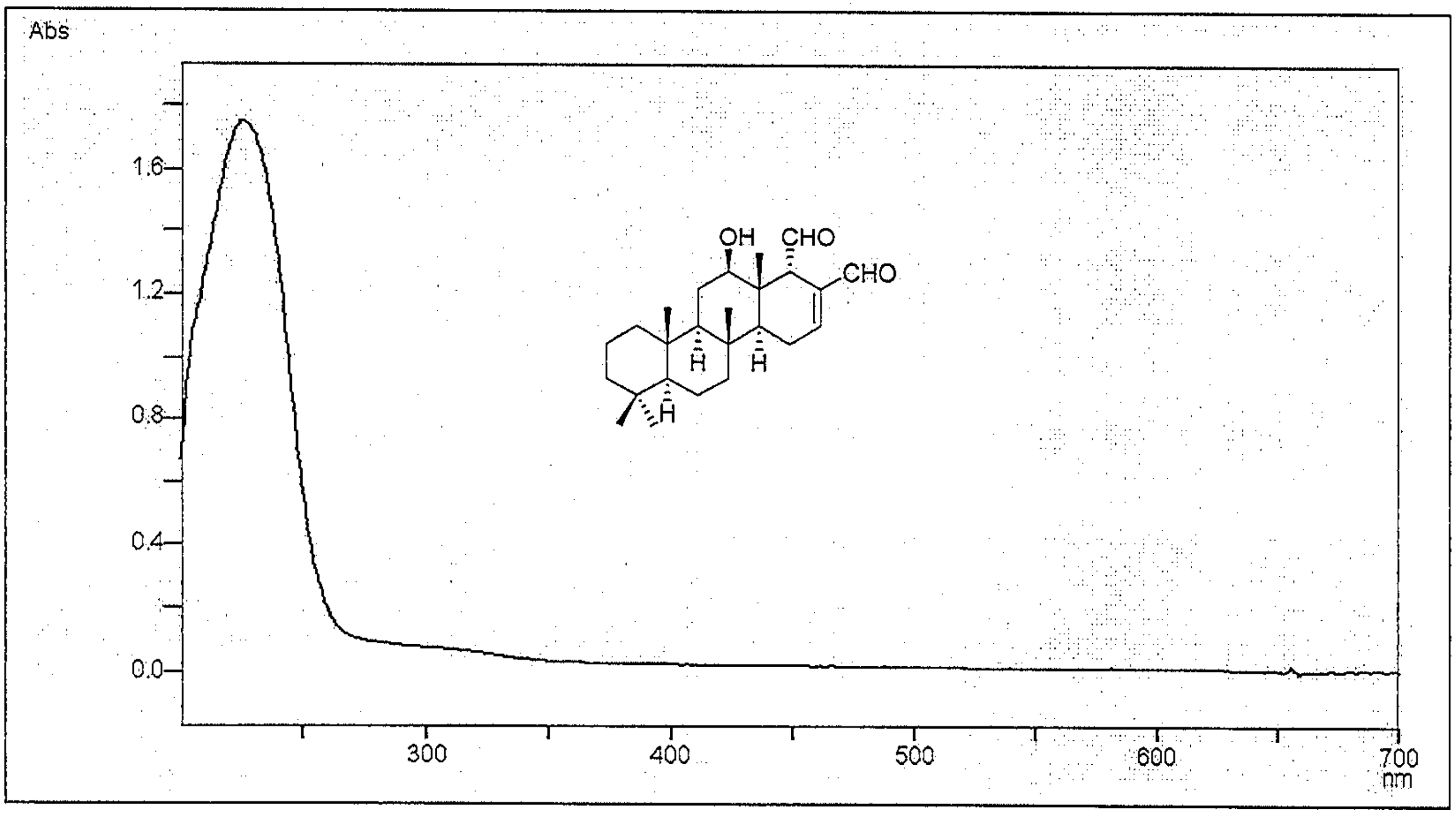
^{1}H NMR spectrum of compound **47** (500 MHz, C_6D_6)



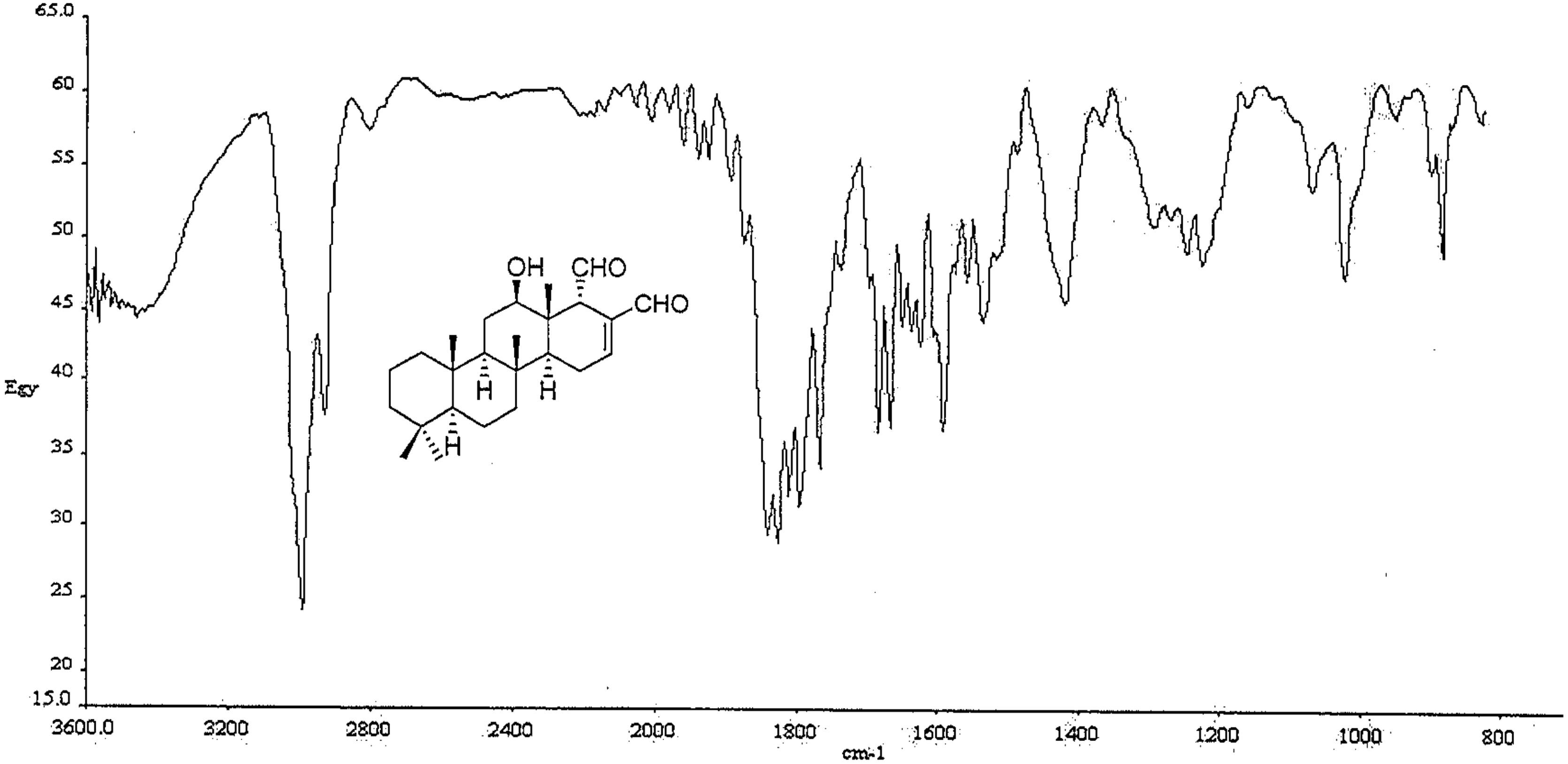
^{13}C NMR spectrum of compound **47** (125 MHz, C_6D_6)



EI mass spectrum of compound 47



UV spectrum of compound **48** (MeOH)



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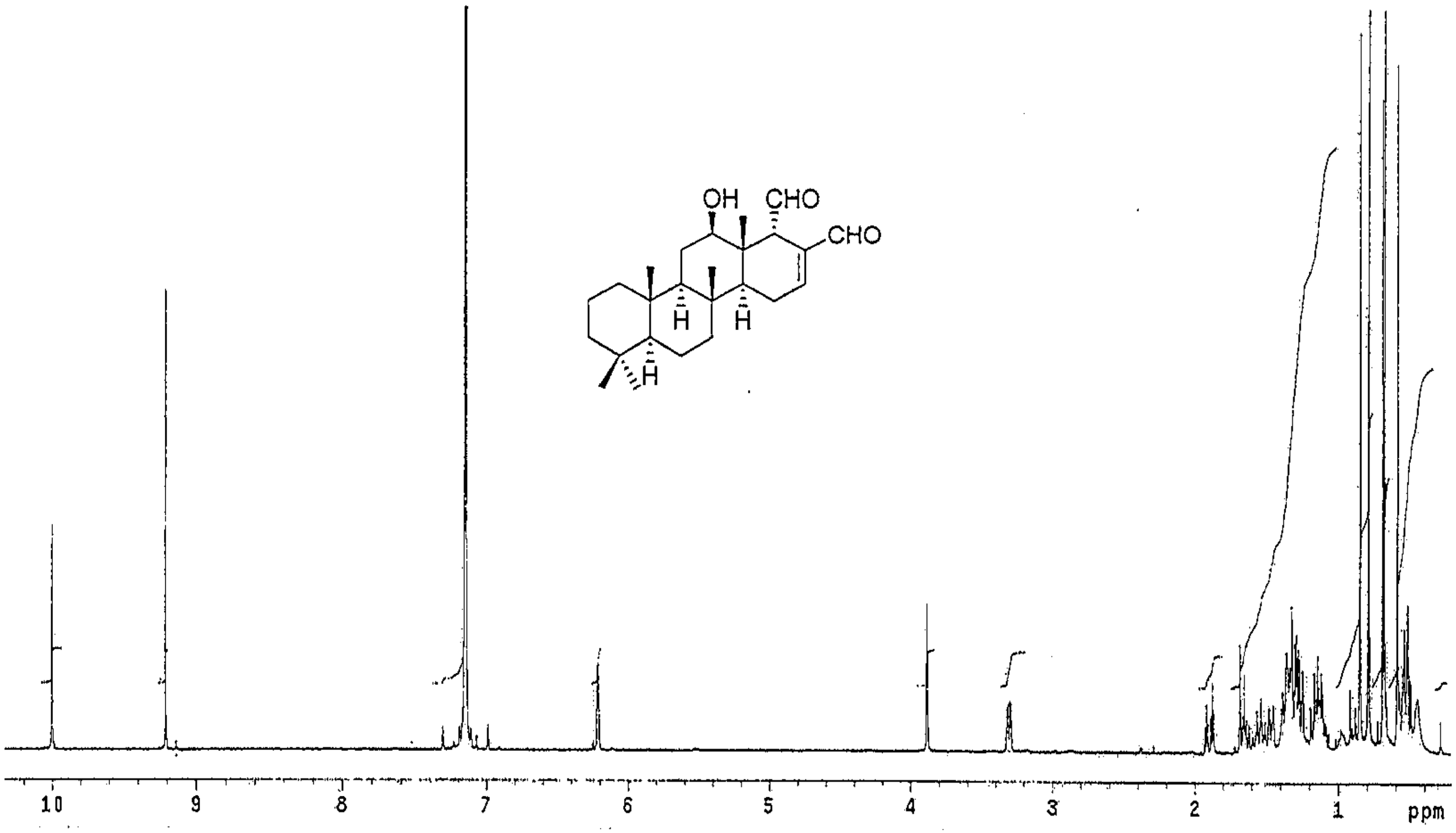

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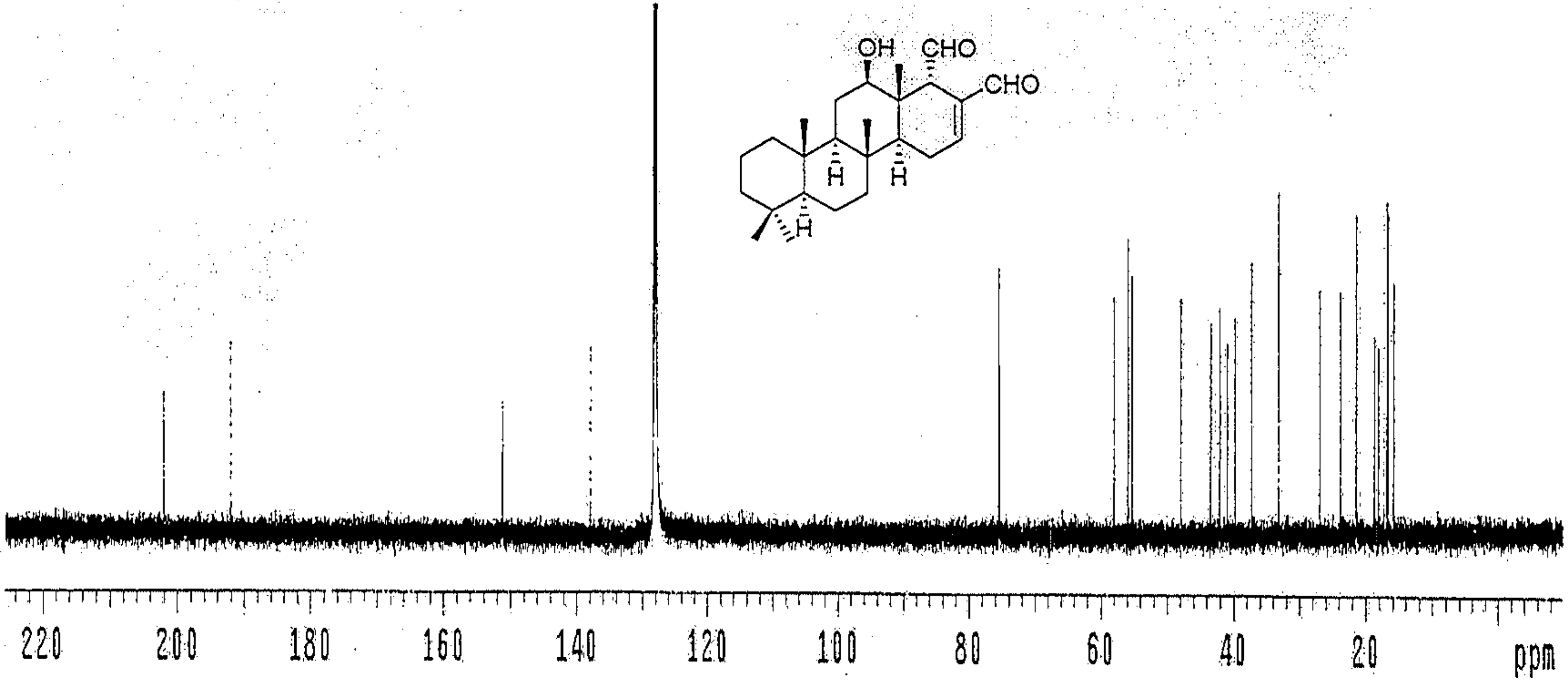
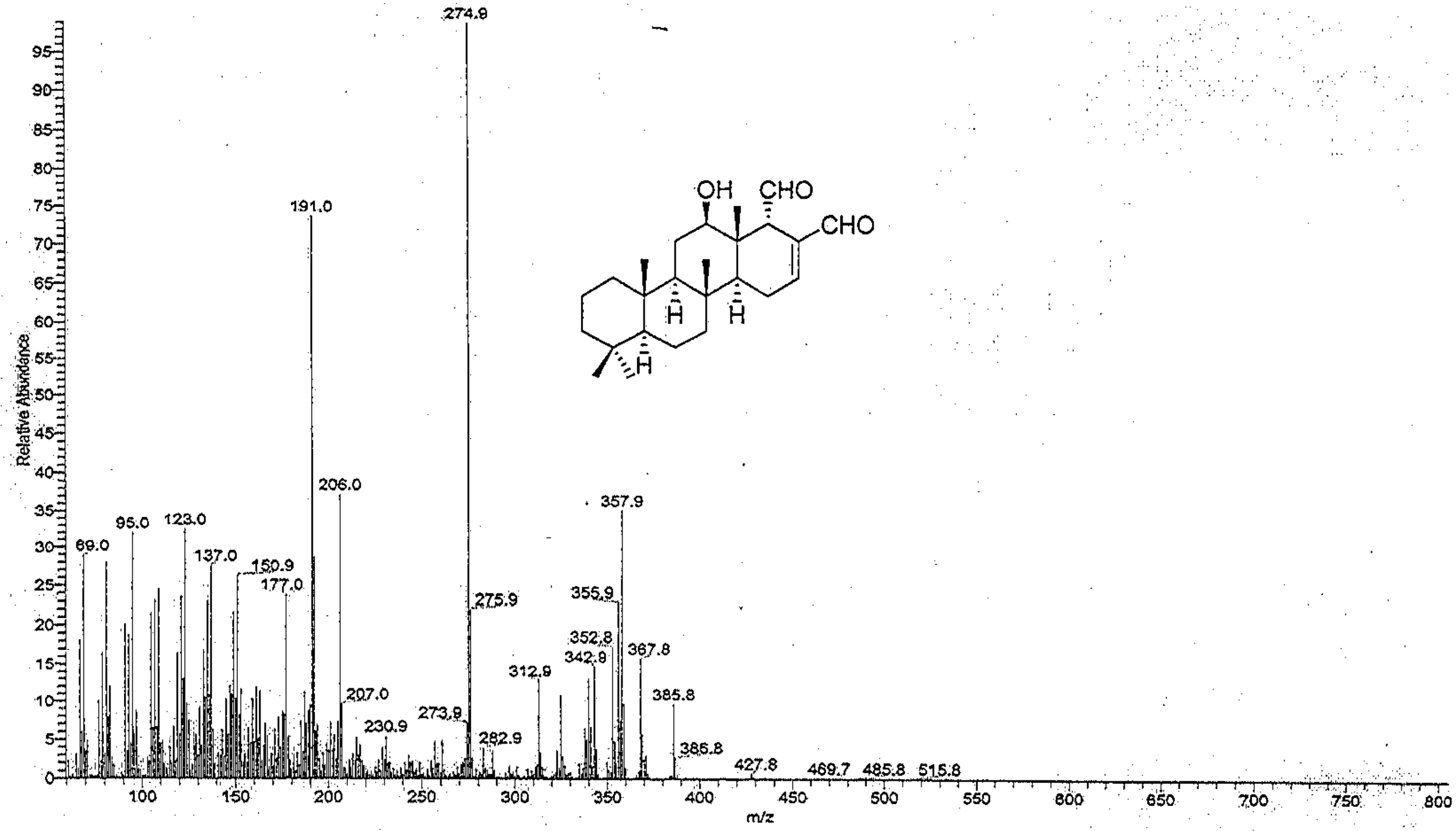


^{13}C NMR spectrum of compound 48 (125 MHz, C_6D_6)



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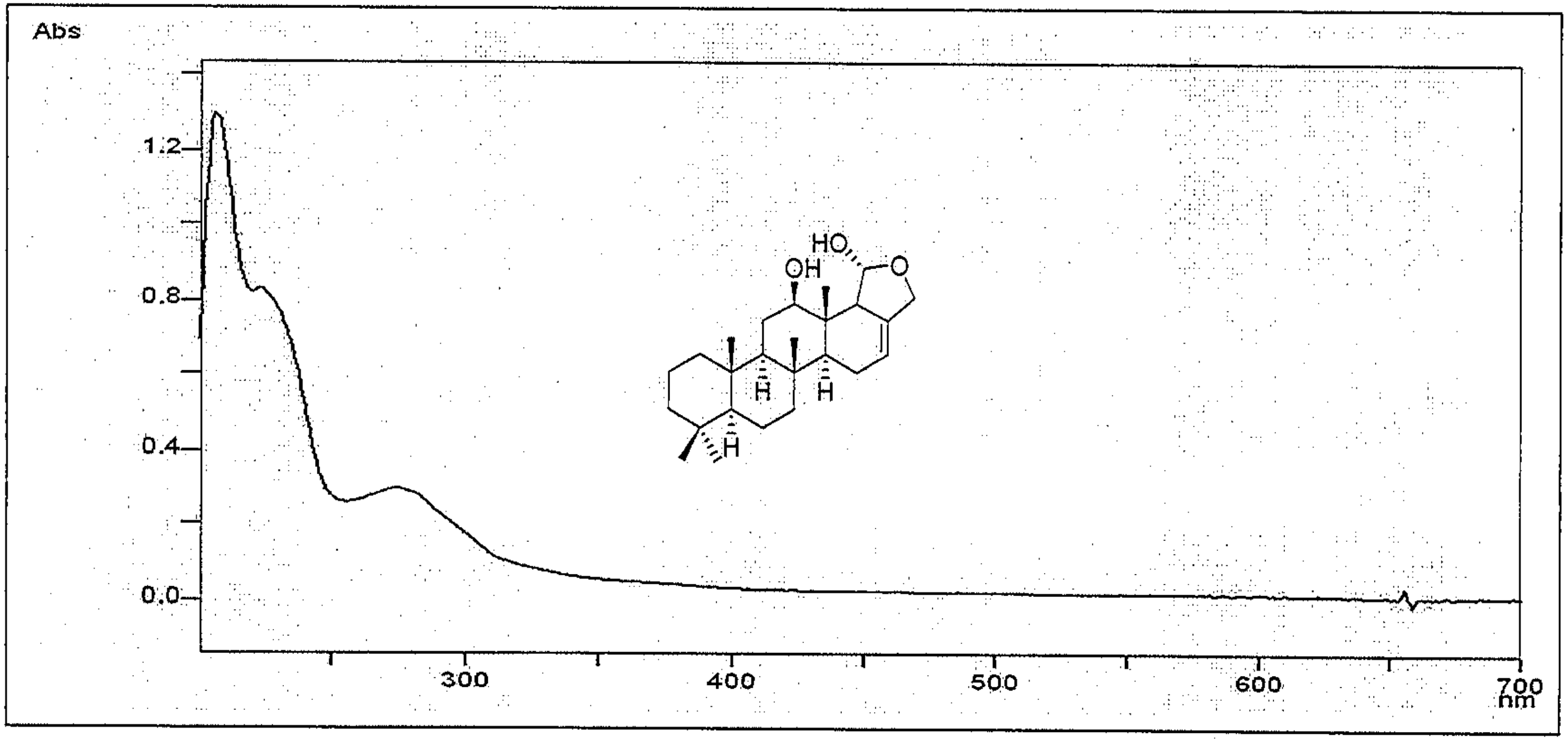

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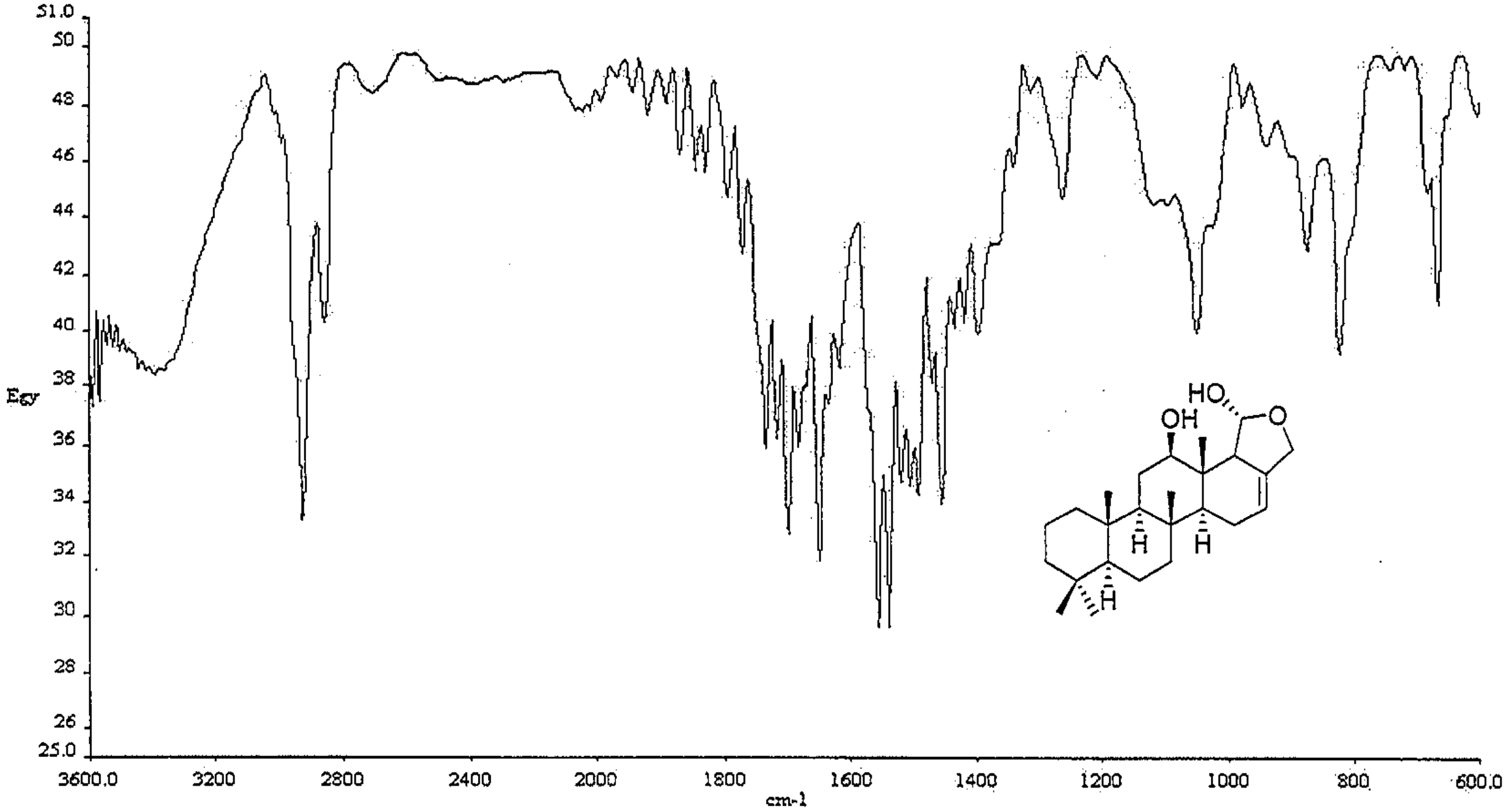
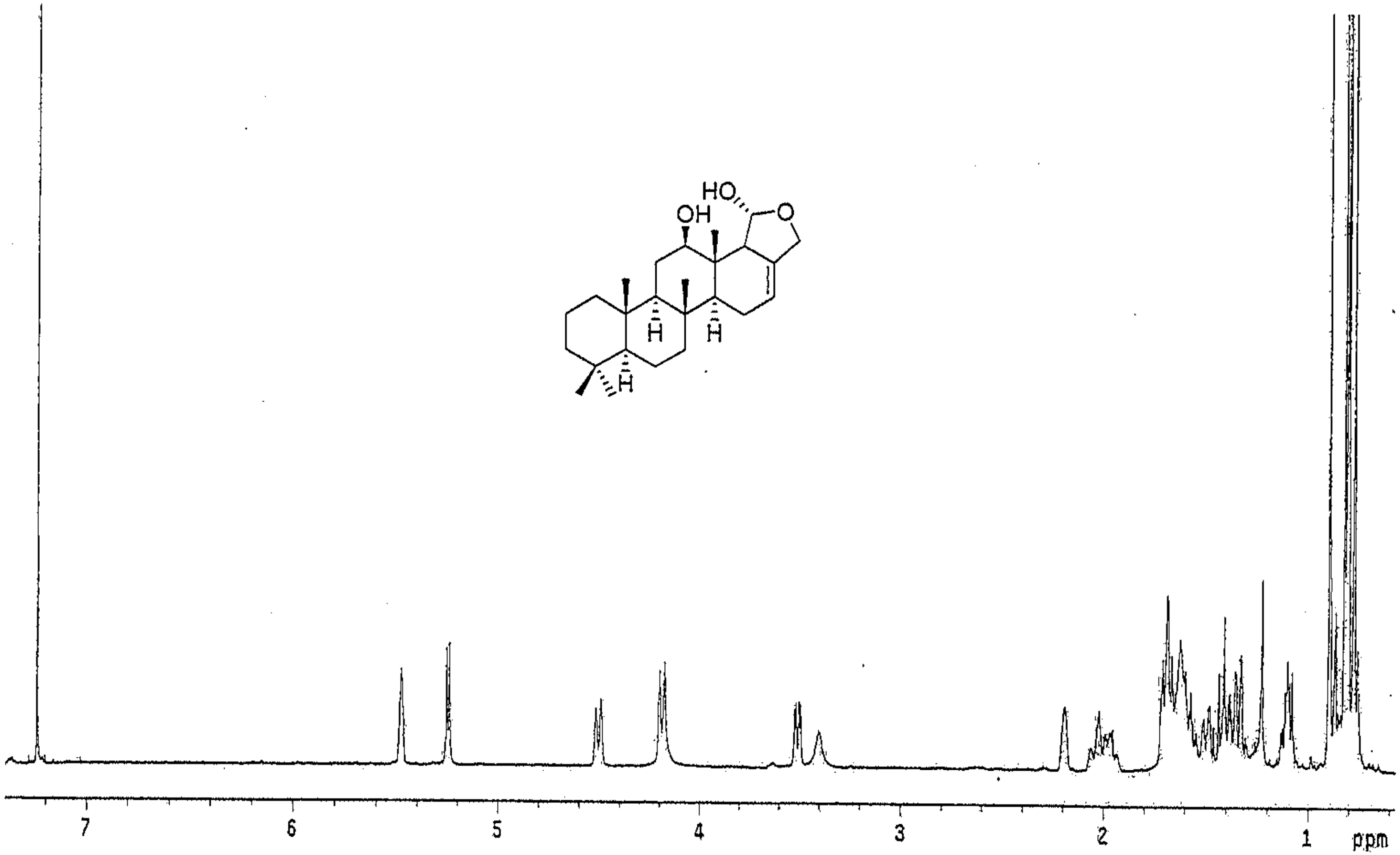


IR spectrum of compound **49** (neat)



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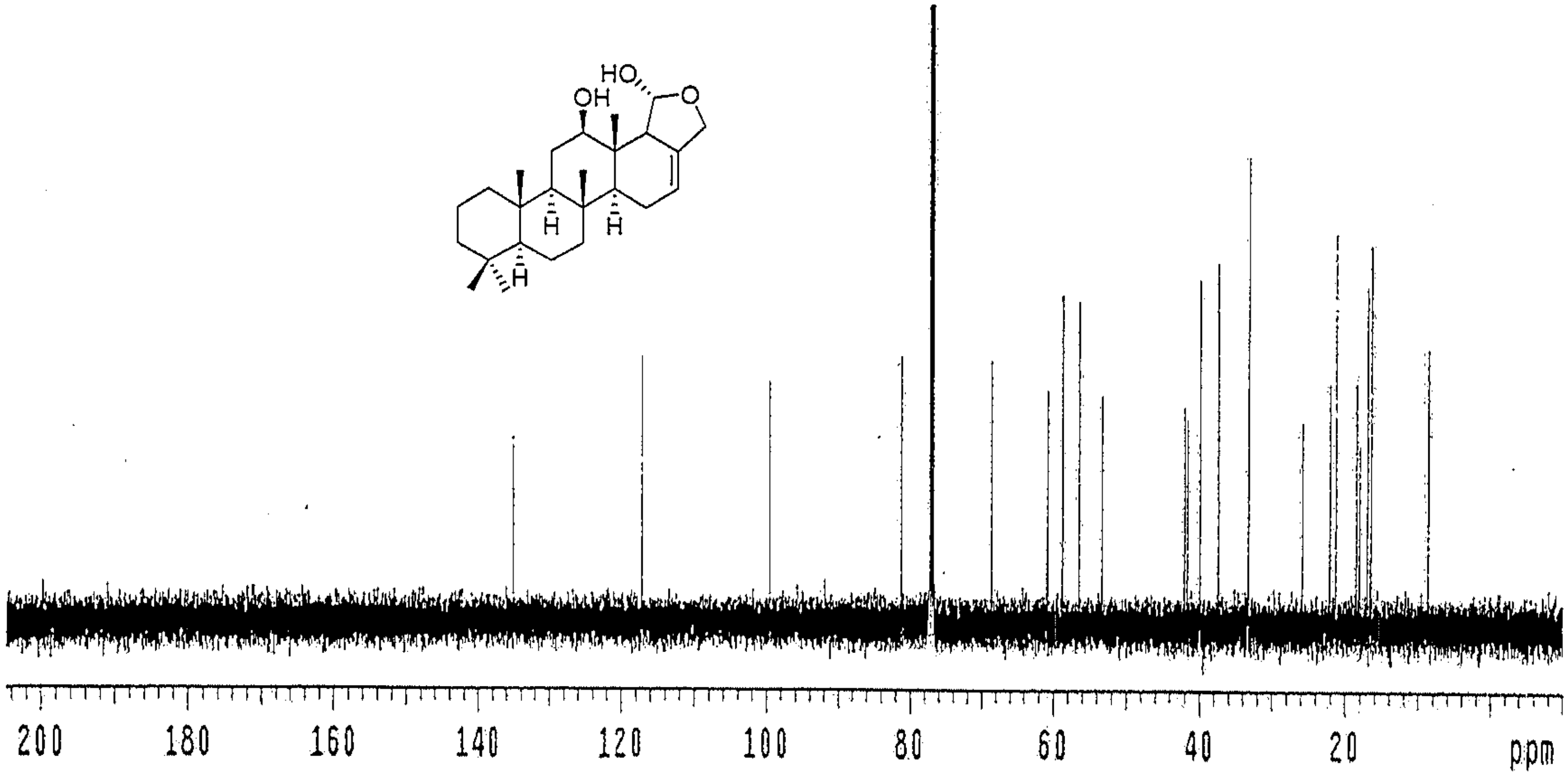

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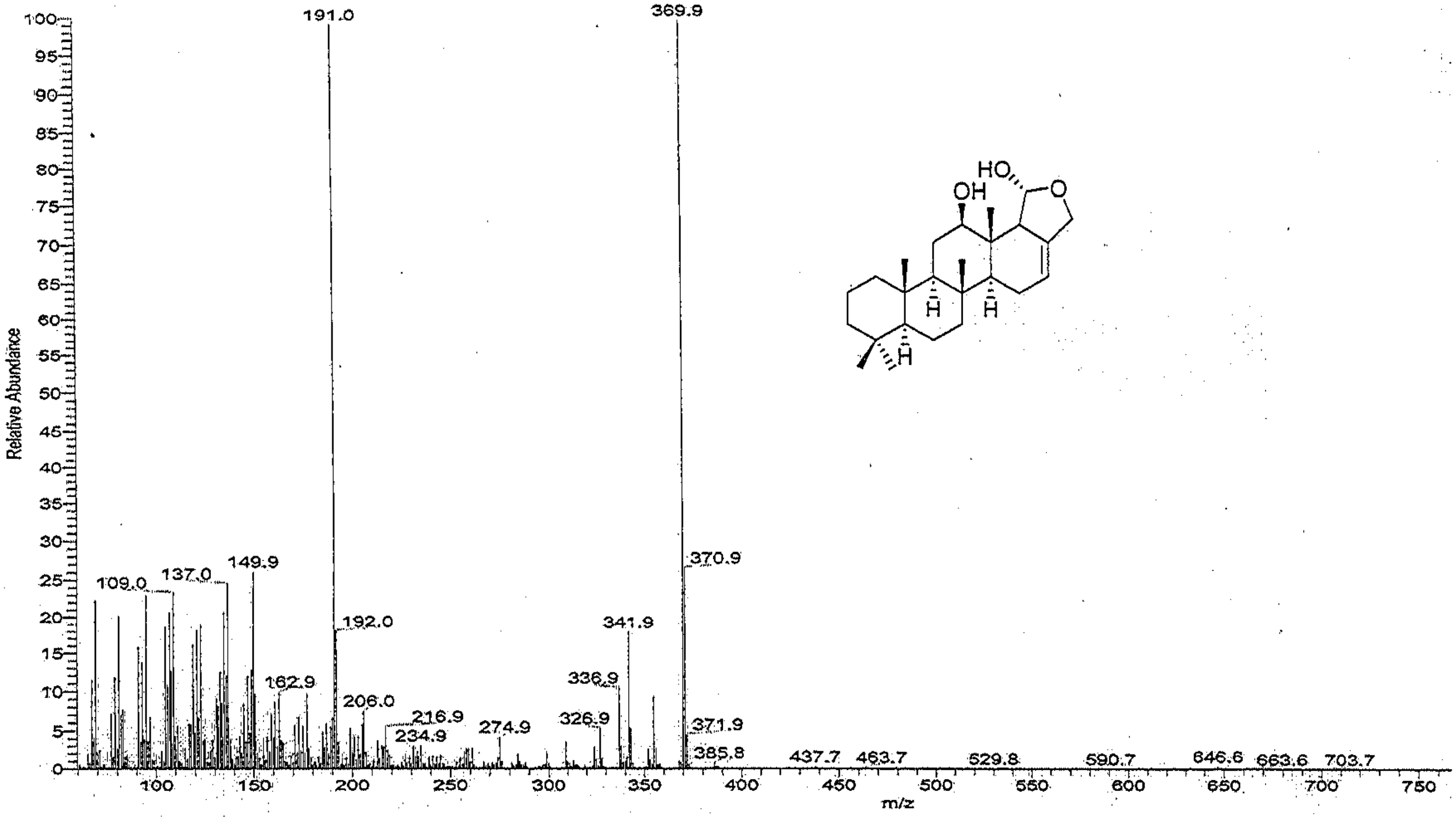
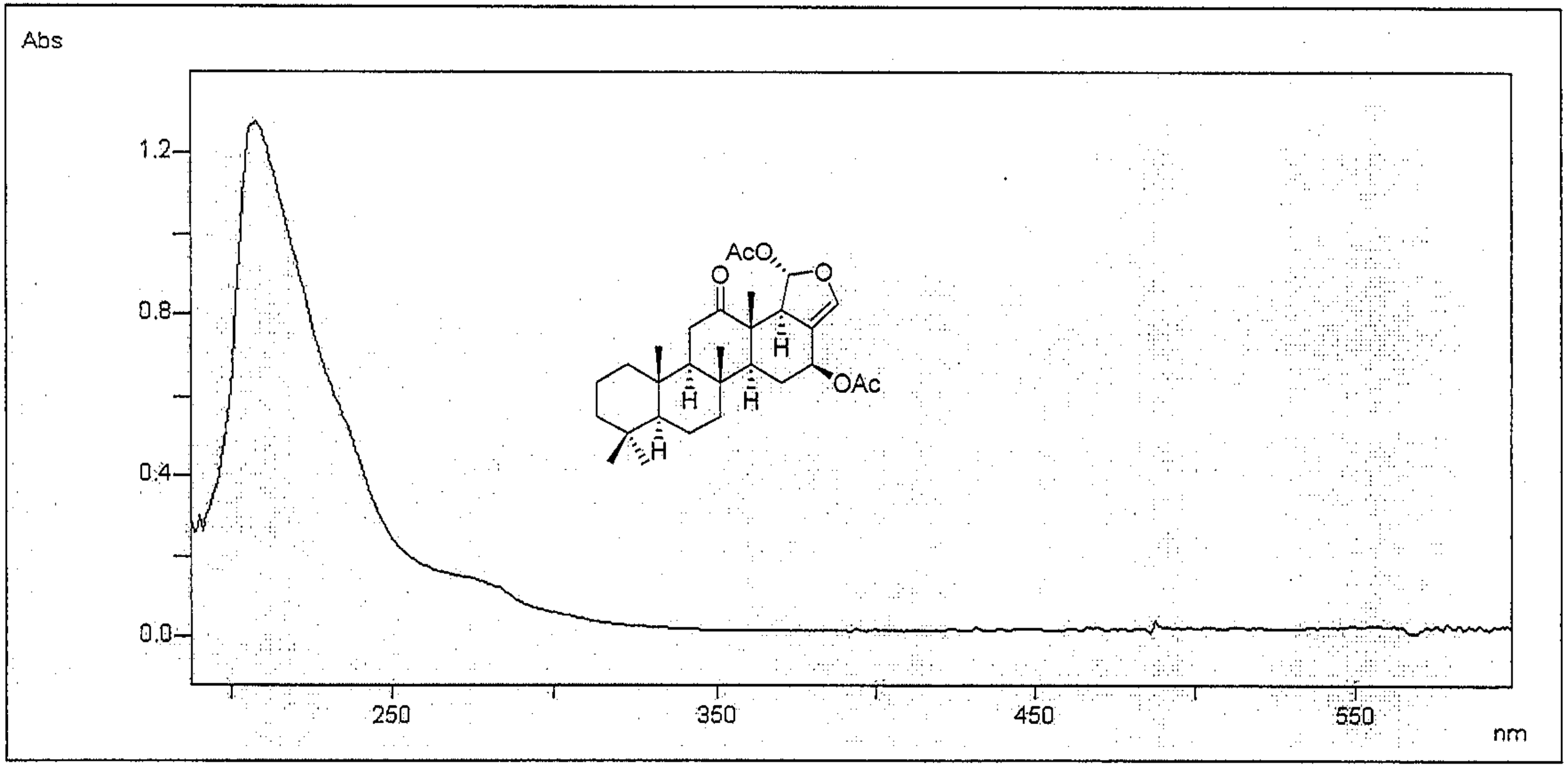


EI mass spectrum of compound 49



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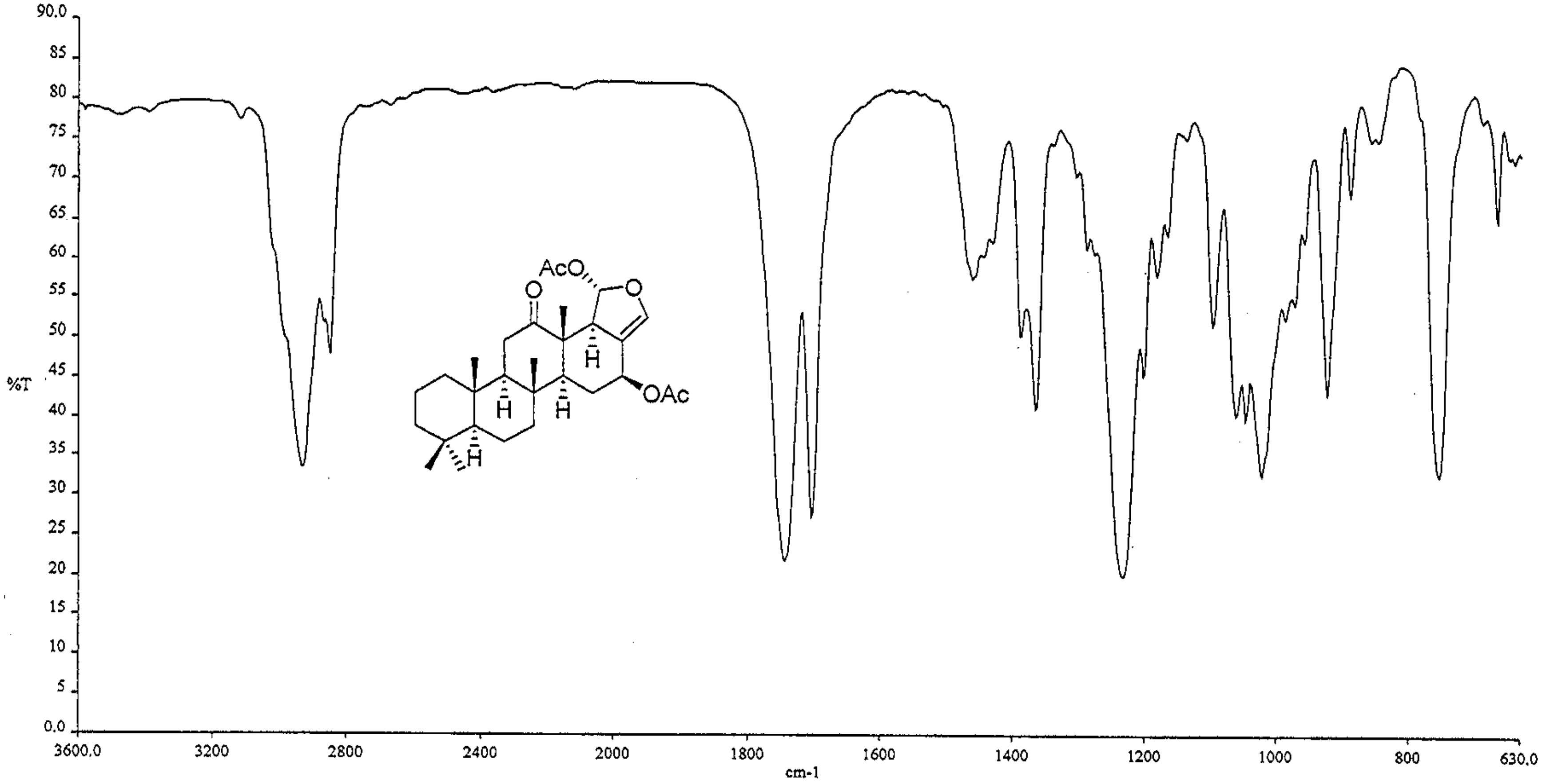
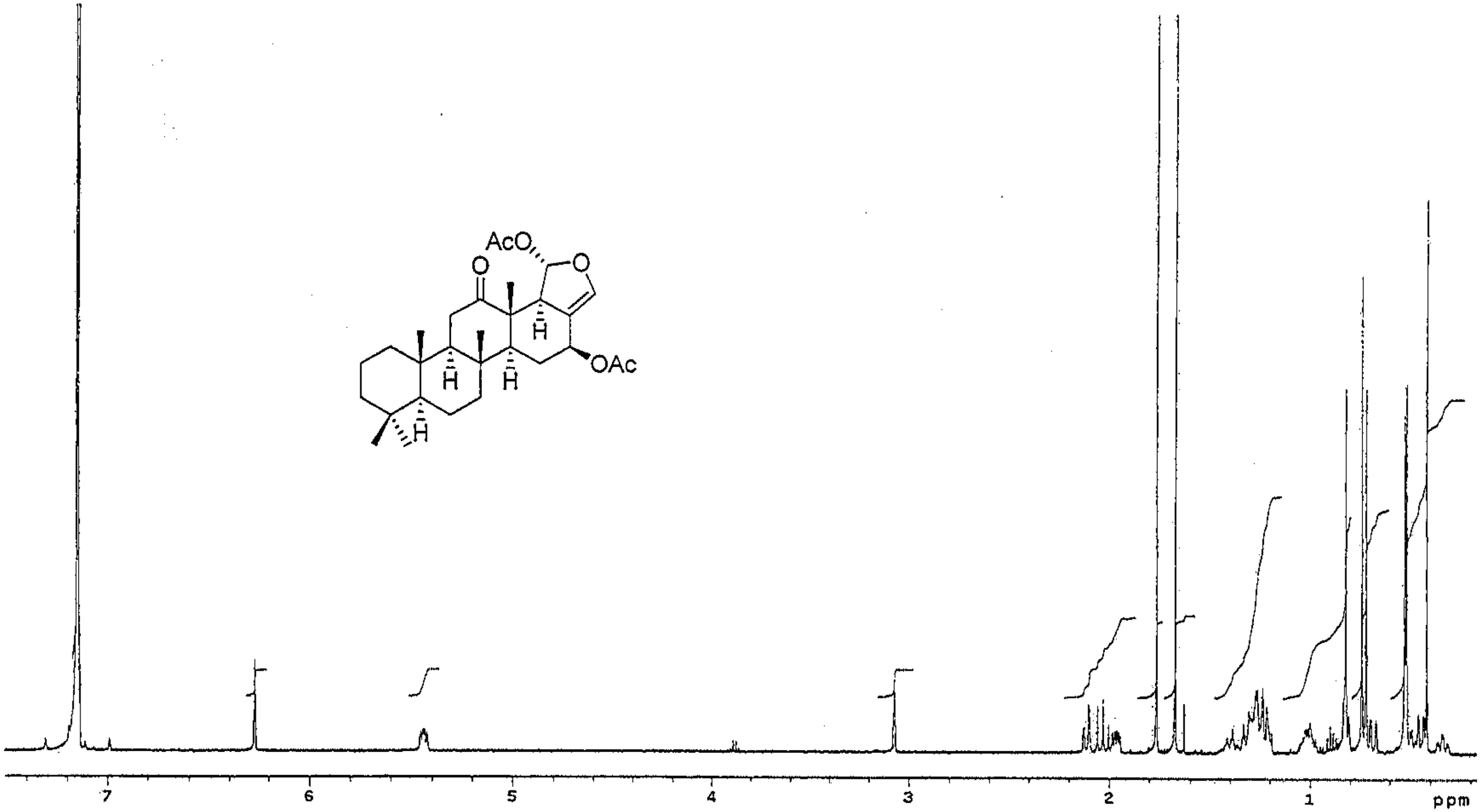
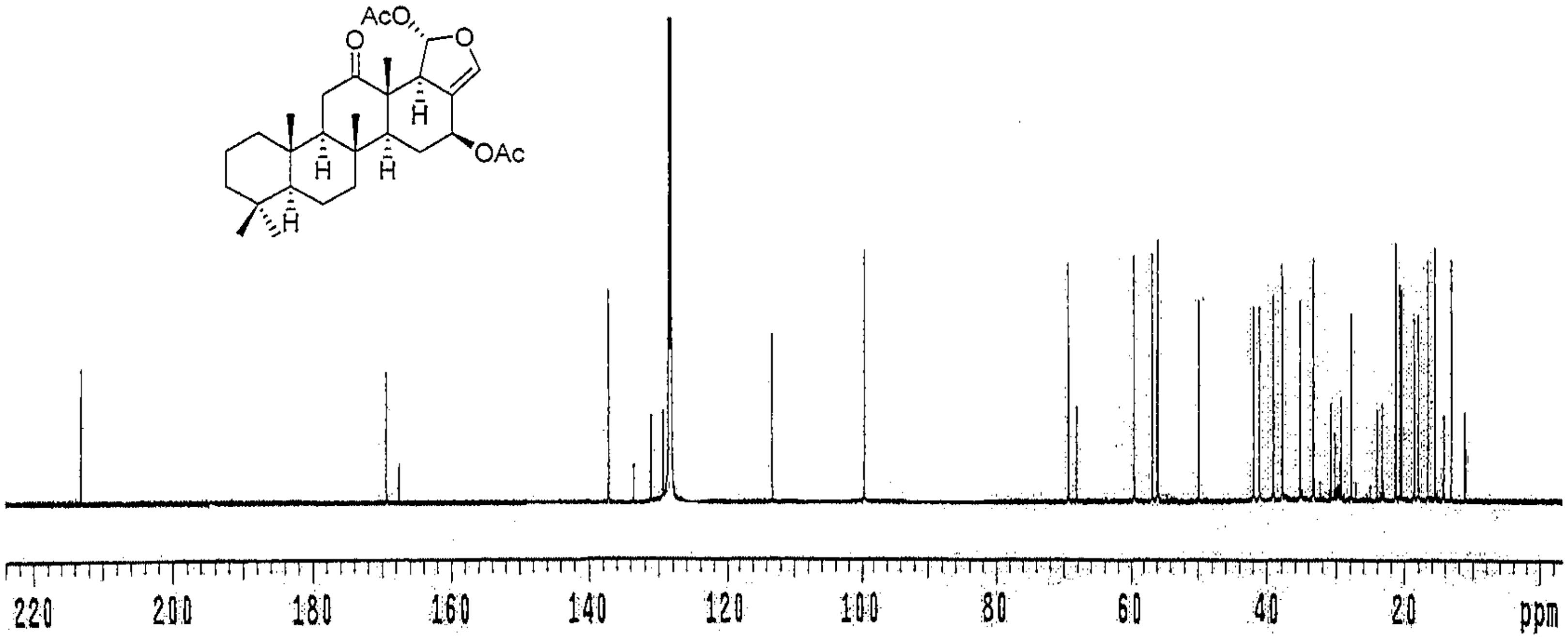


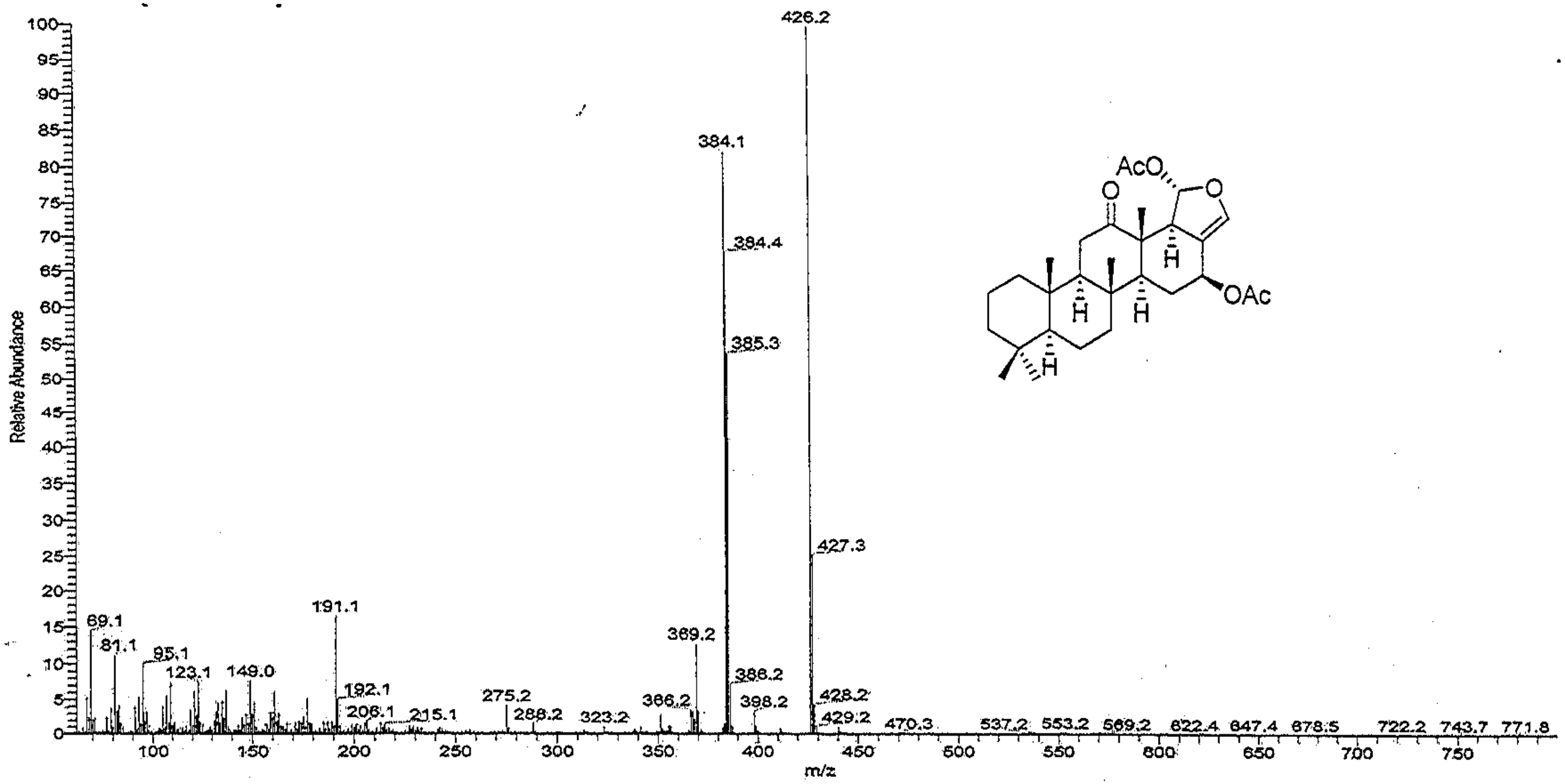
IR spectrum of compound **50** (neat)



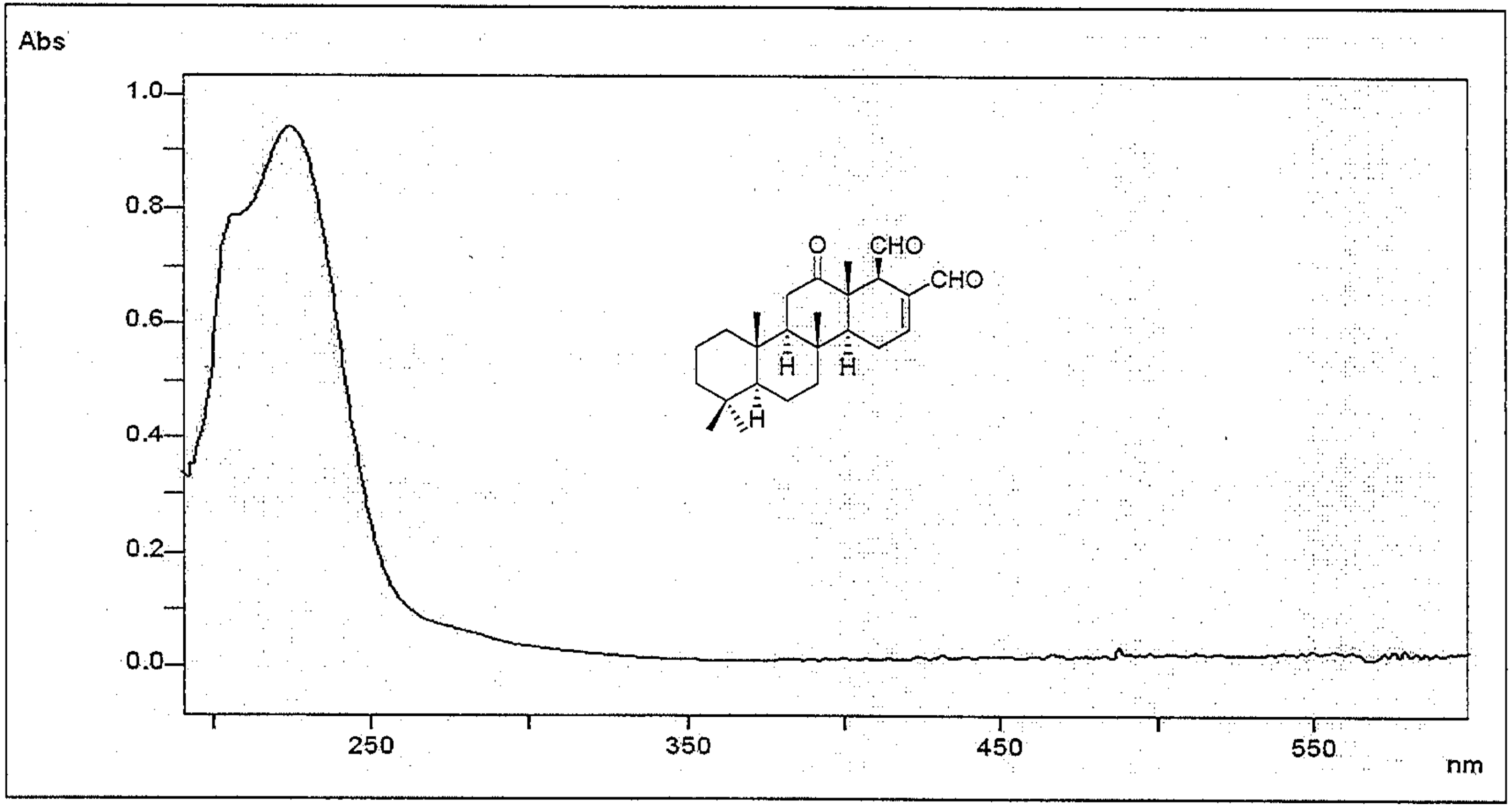
^{1}H NMR spectrum of compound **50** (500 MHz, C_6D_6)



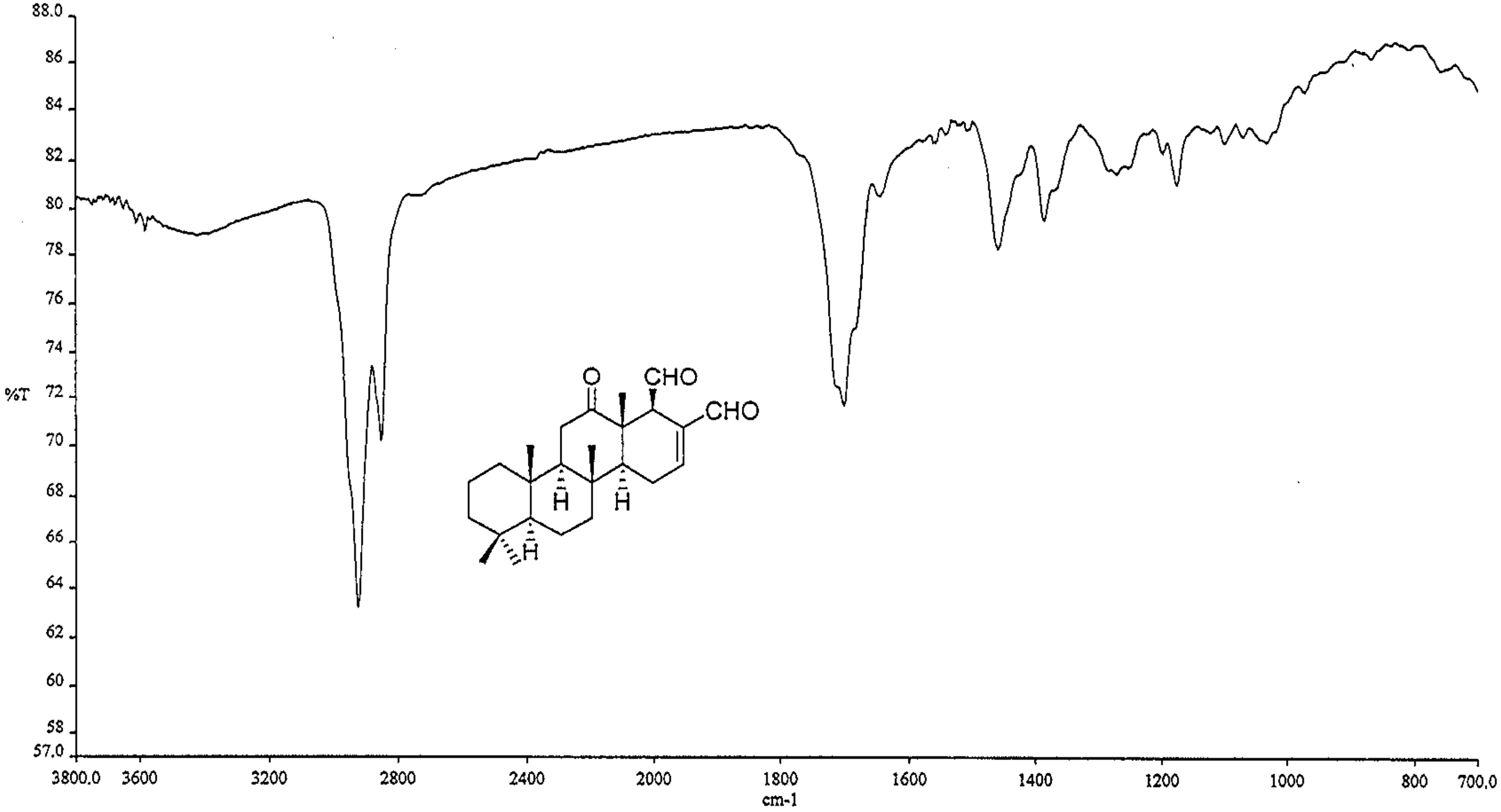
^{13}C NMR spectrum of compound 50 (125 MHz), C_6D_6)



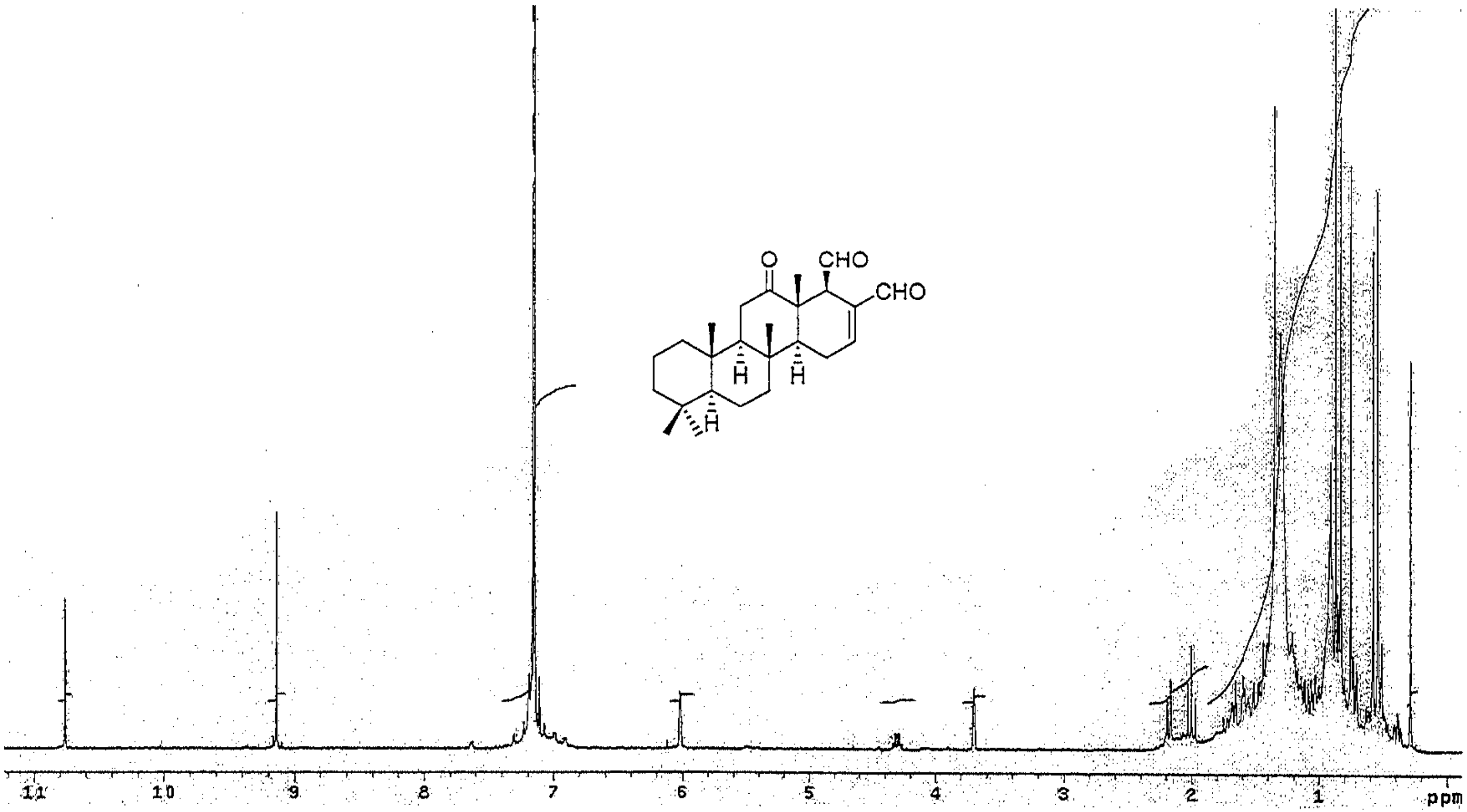
EI mass spectrum of compound 50



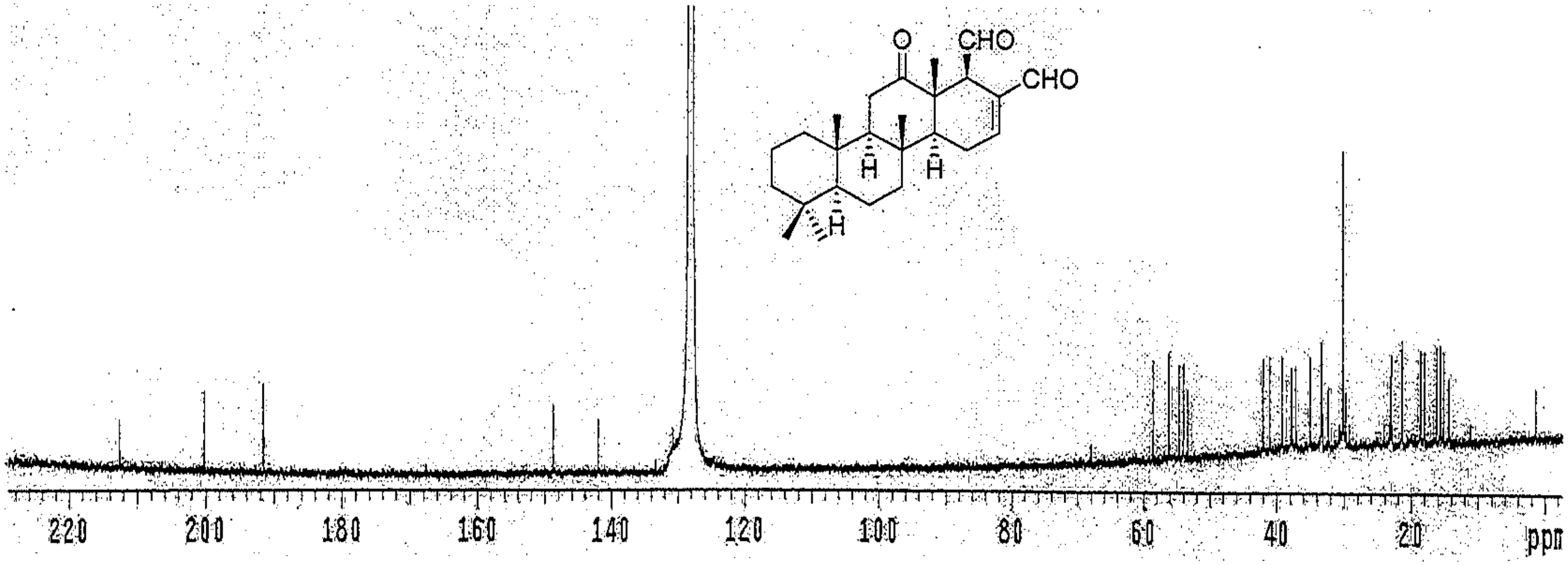
UV spectrum of compound **51** (MeOH)



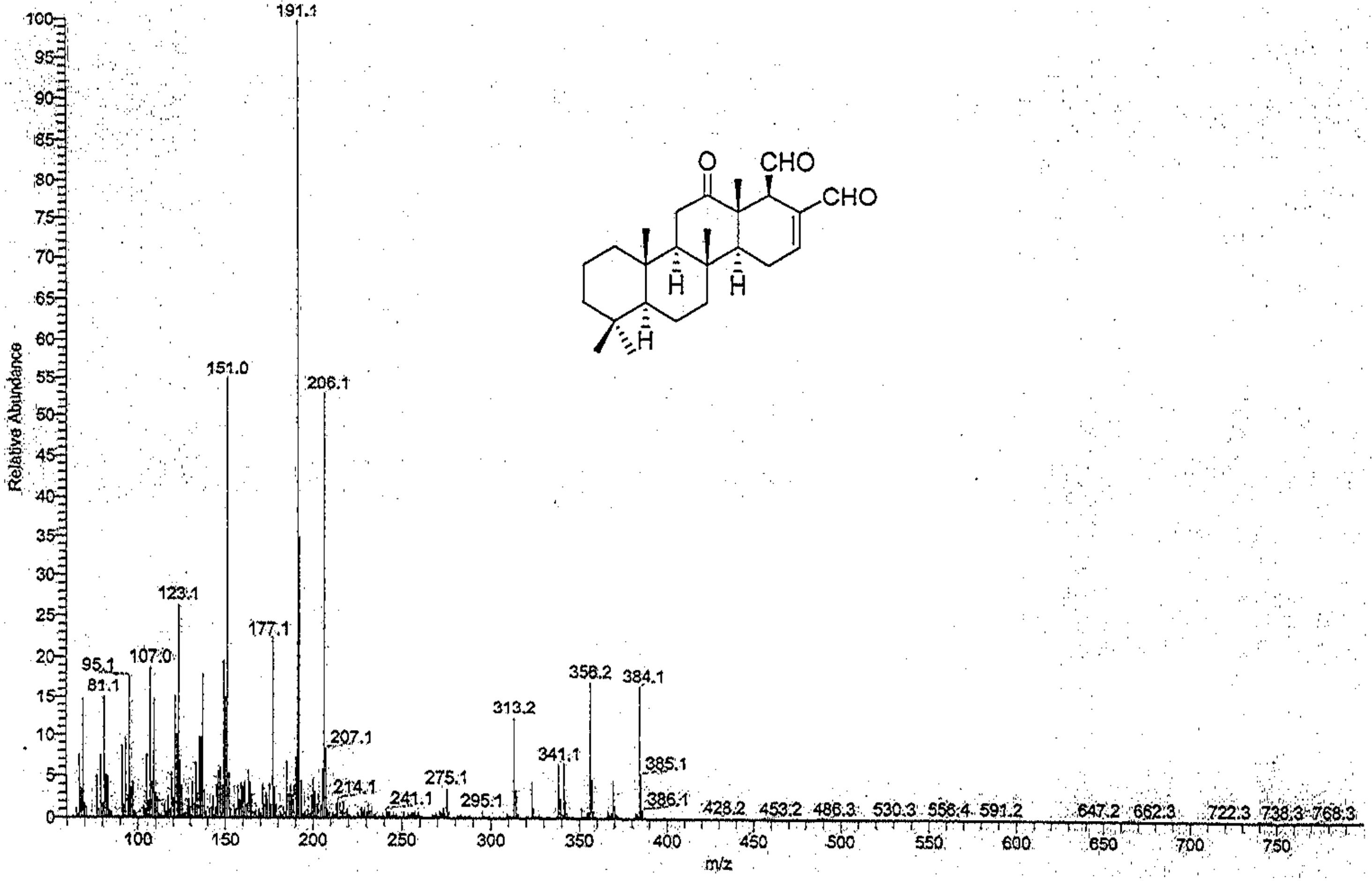
IR spectrum of compound **51** (neat)



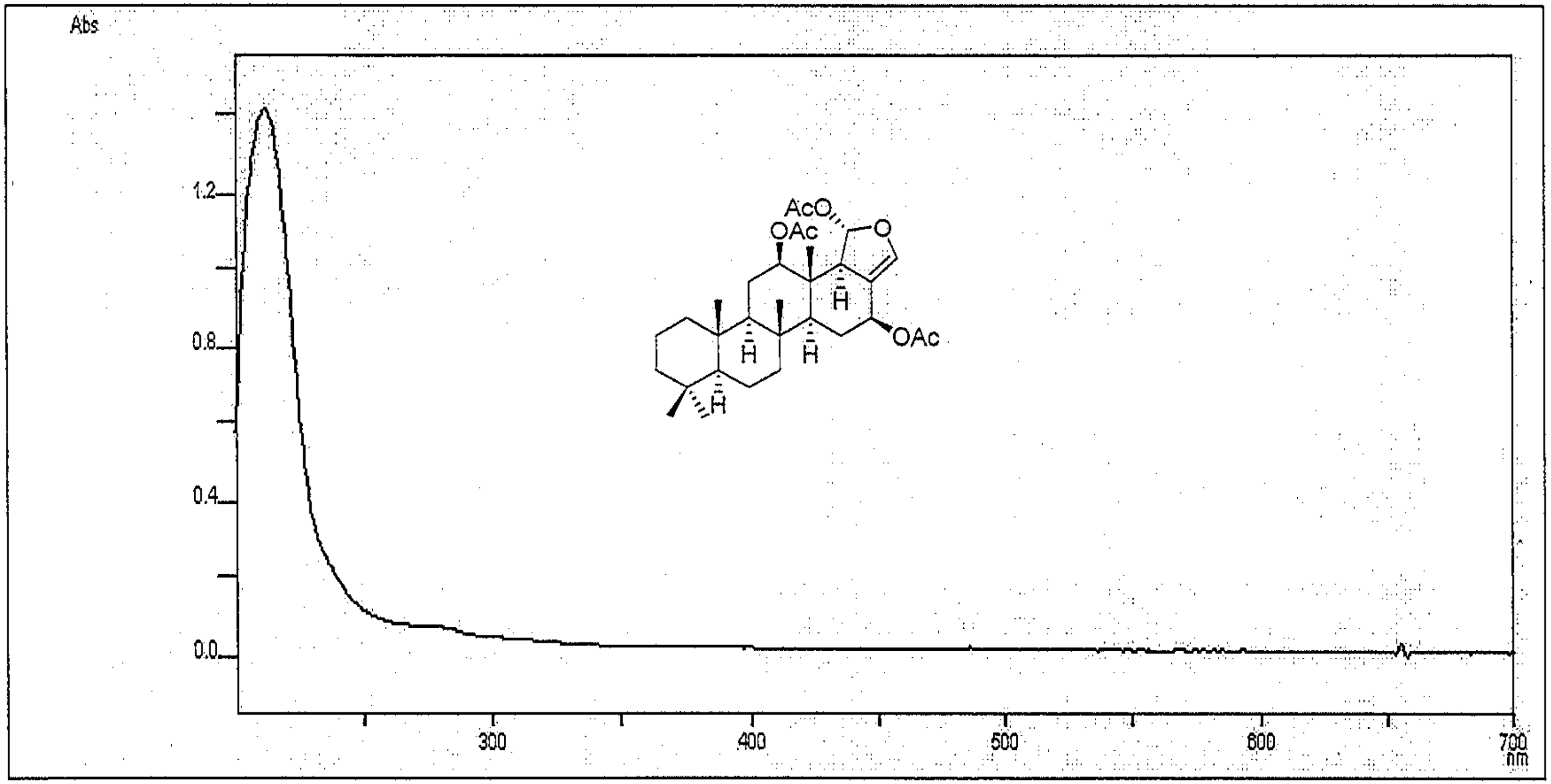
^{1}H NMR spectrum of compound 51 (500 MHz, C_6D_6)



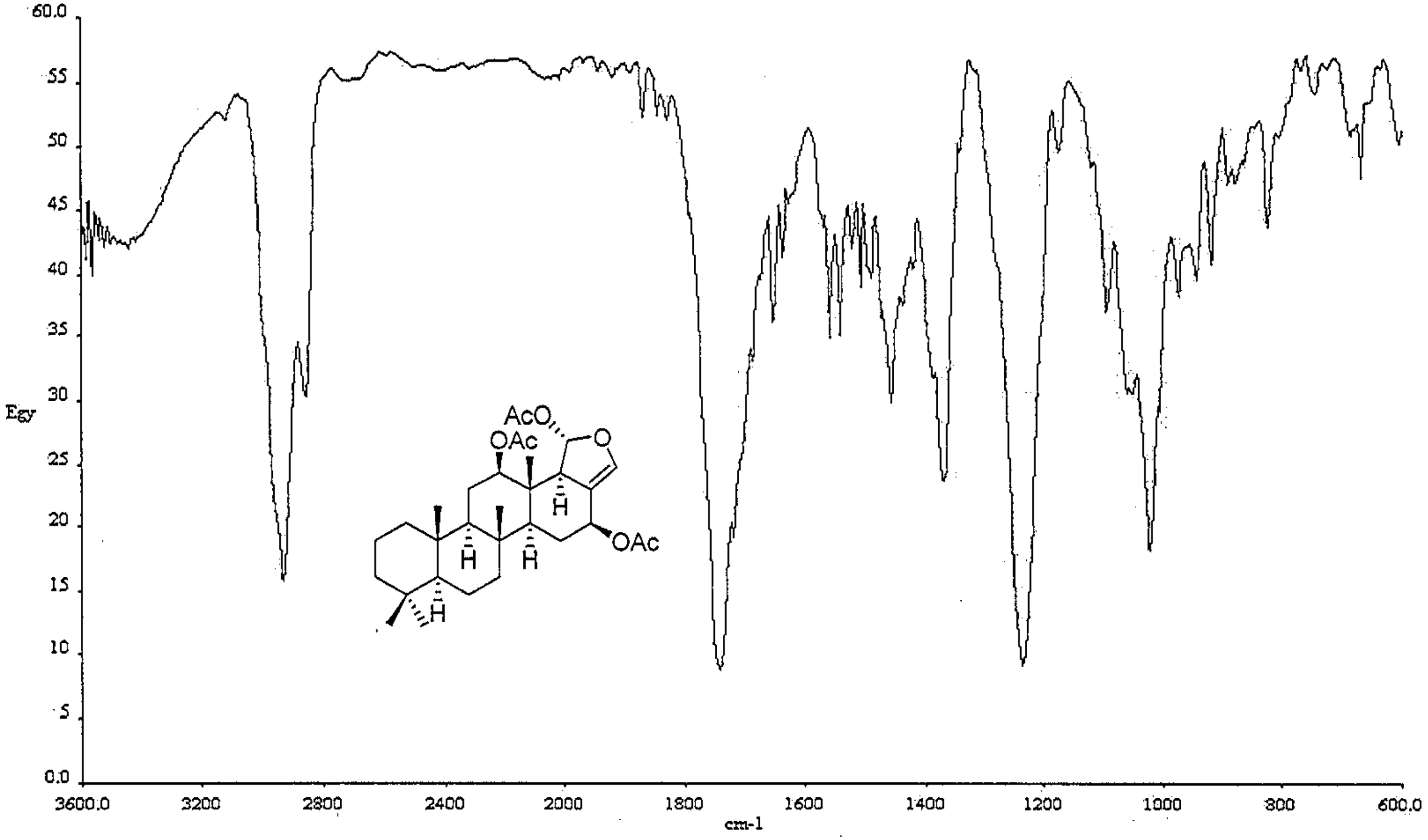
^{13}C NMR spectrum of compound **51** (125 MHz, C_6D_6)



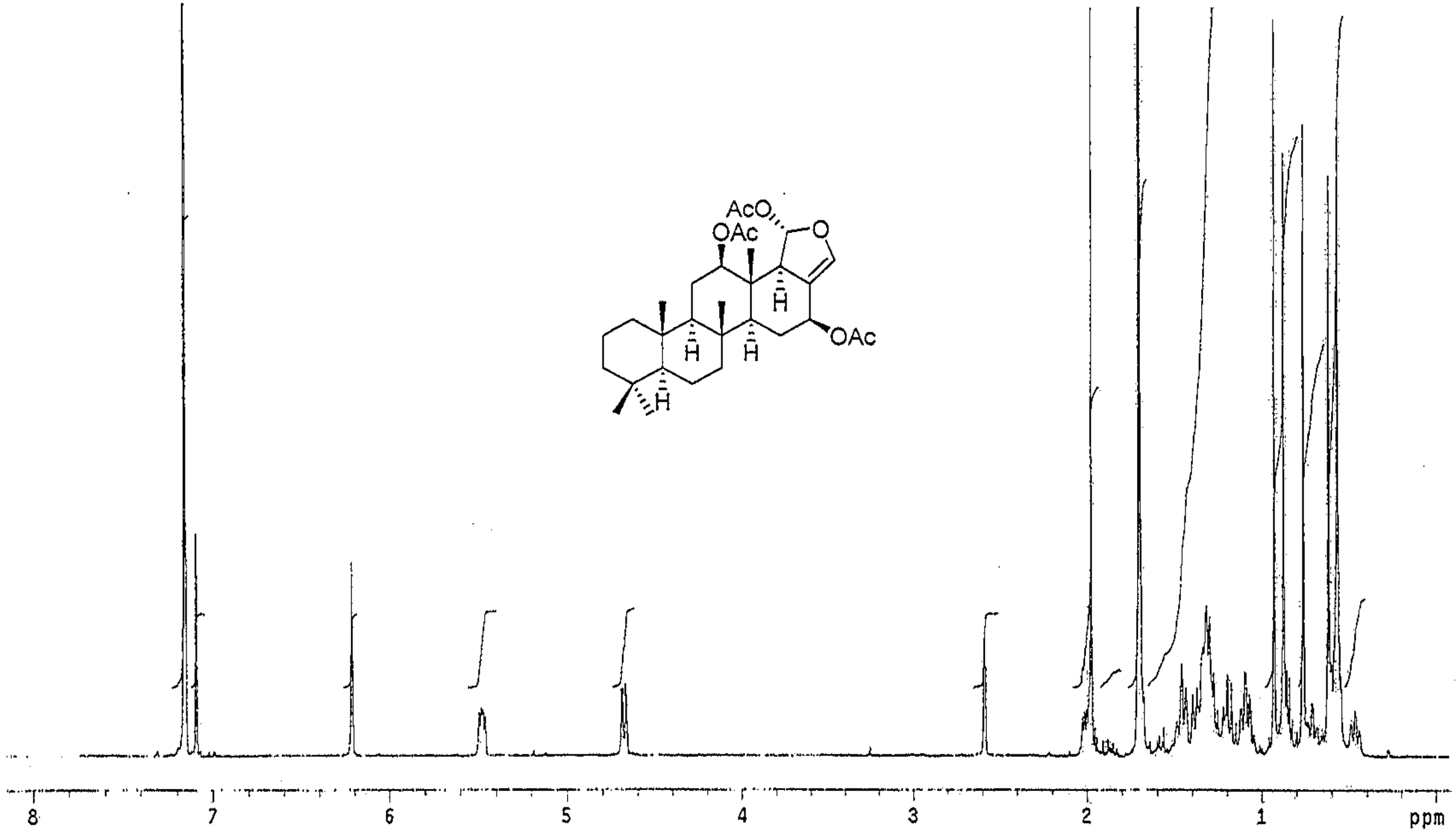
EI mass spectrum of compound 51



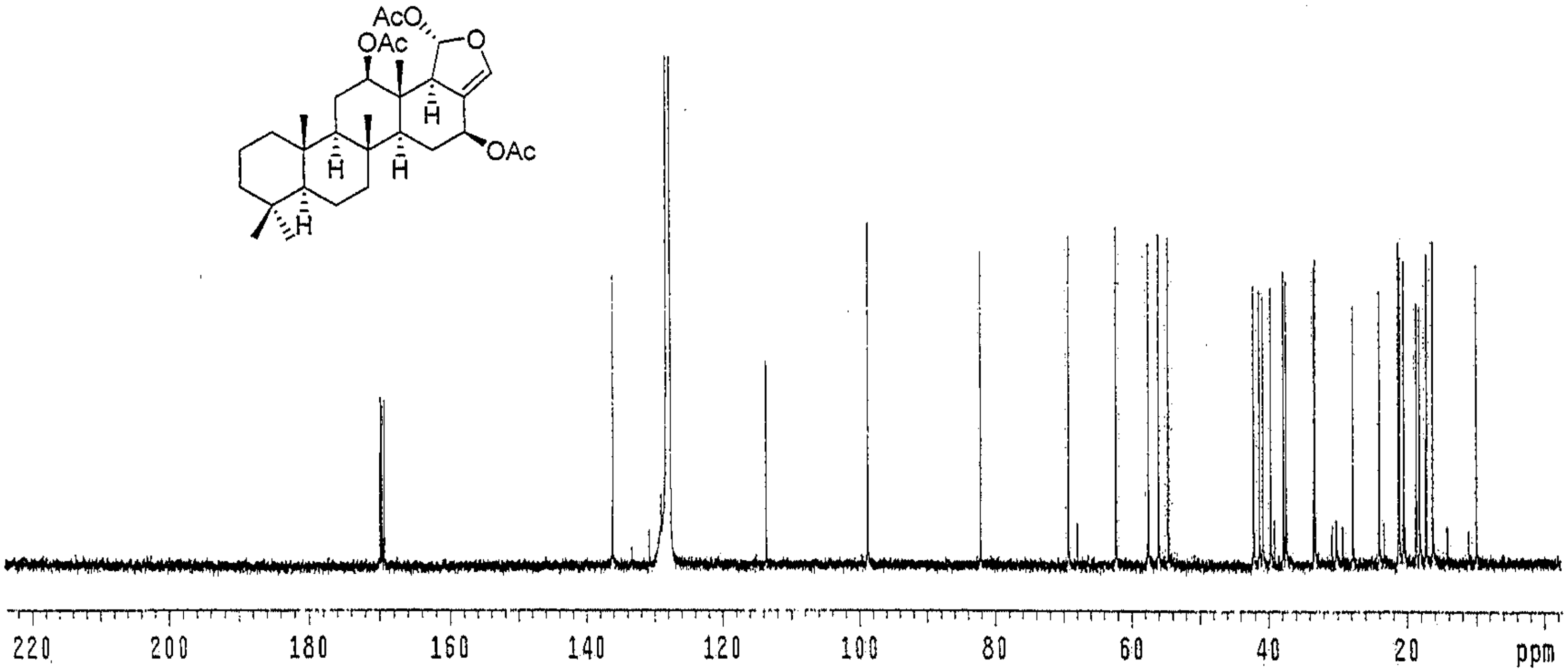
UV spectrum of compound 39 (MeOH)



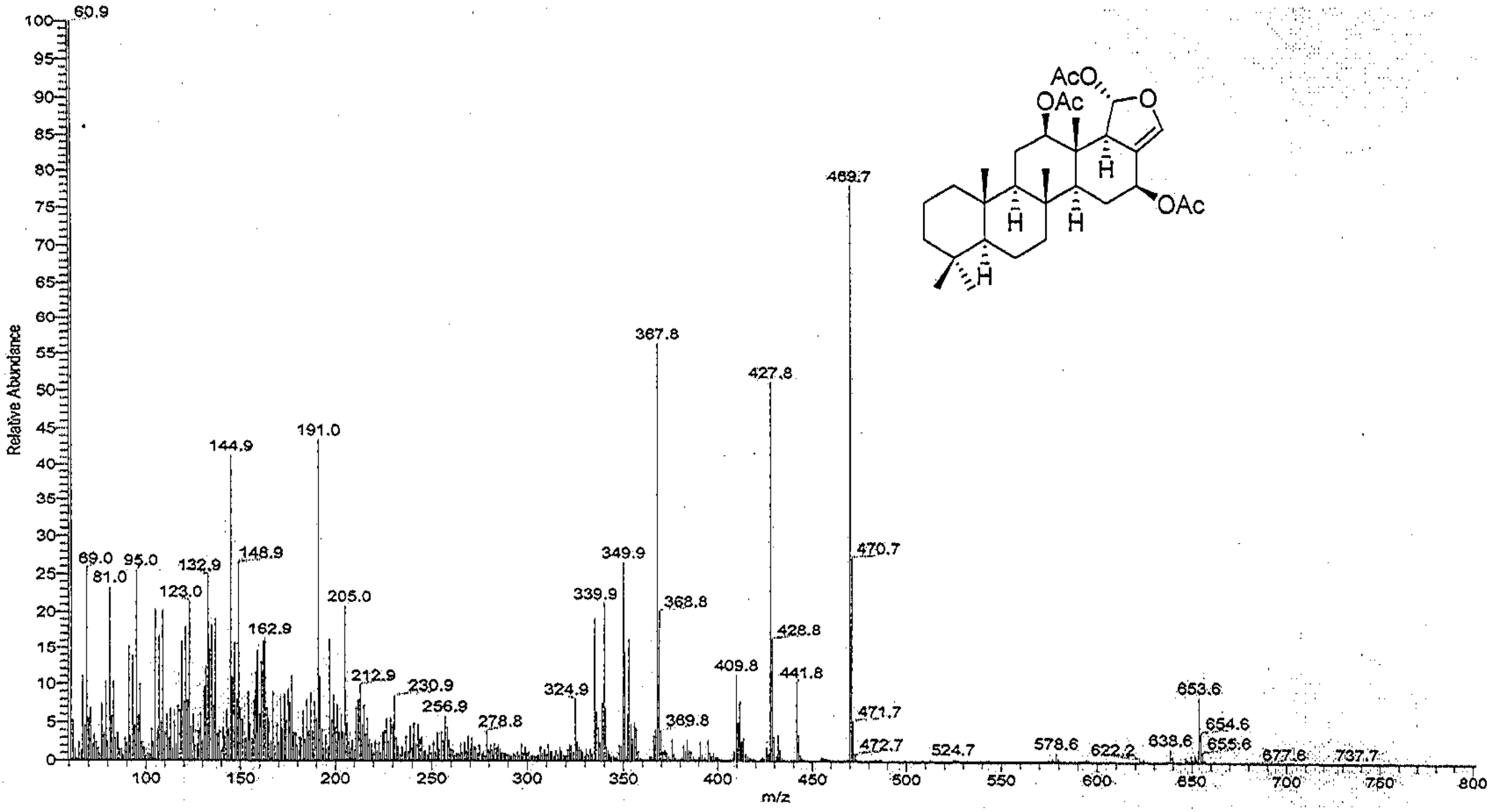
IR spectrum of compound 39 (neat)



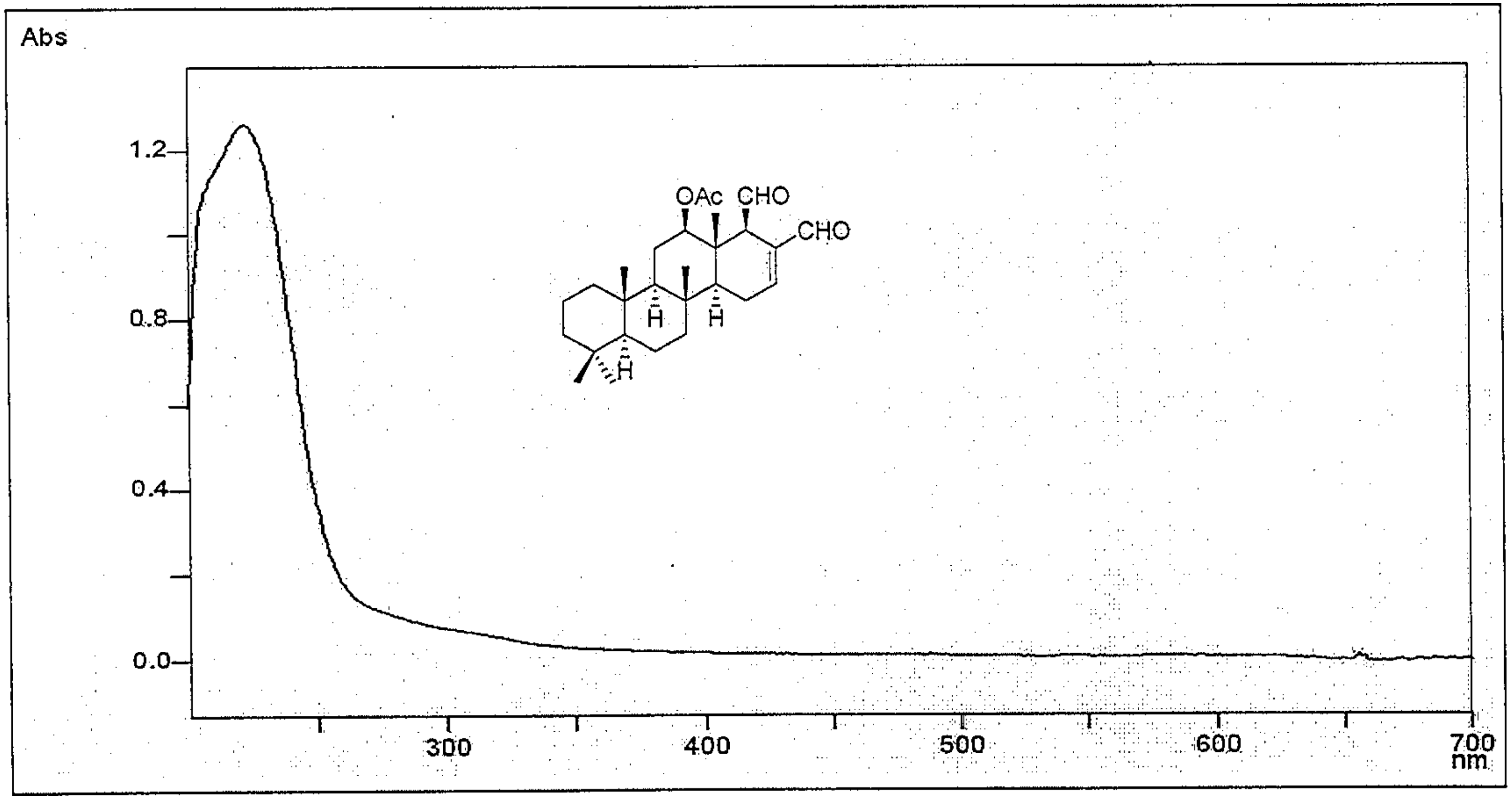
^{1}H NMR spectrum of compound 39 (500 MHz, C_6D_6)



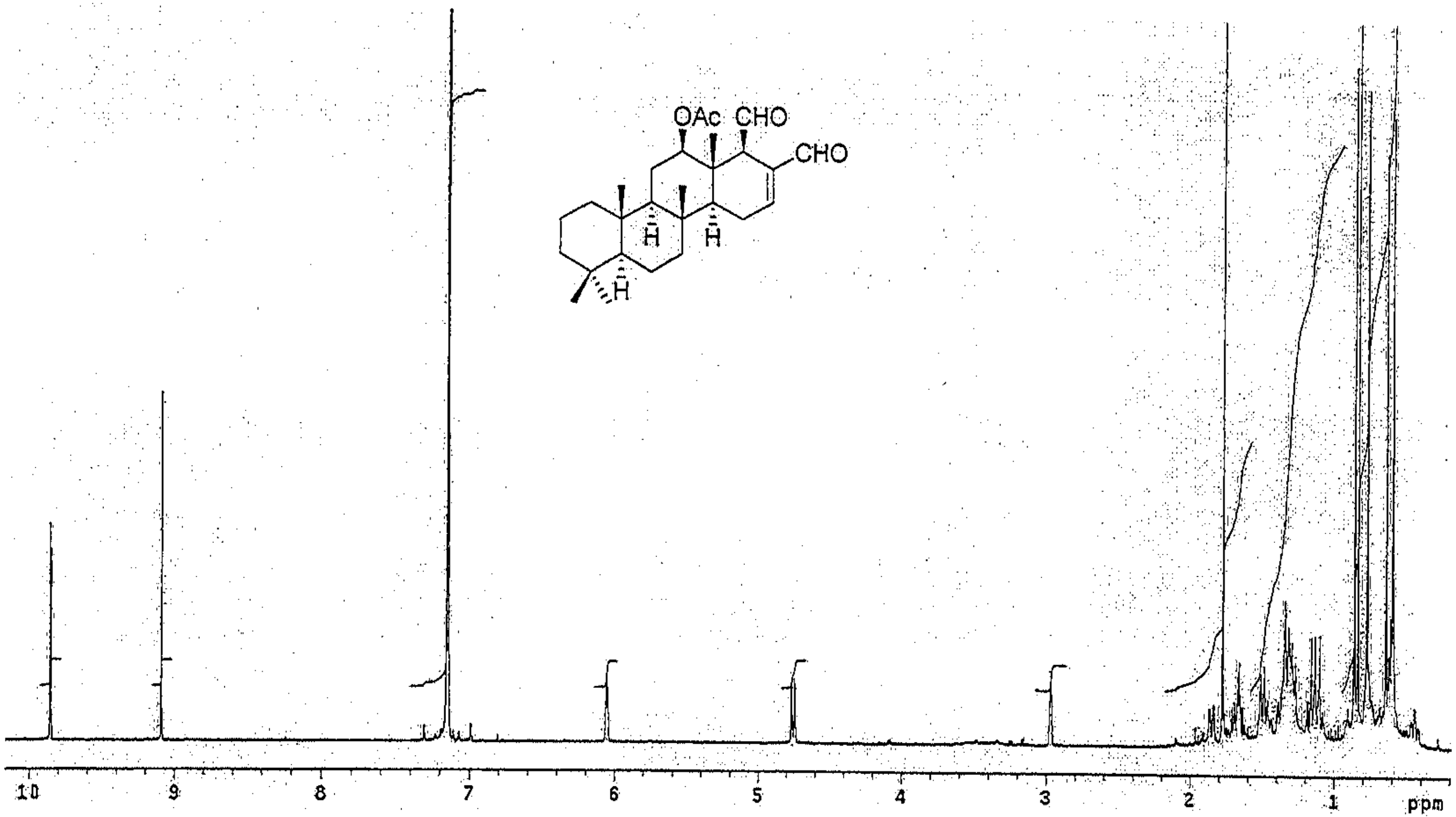
^{13}C NMR spectrum of compound 39 (125 MHz, C_6D_6)



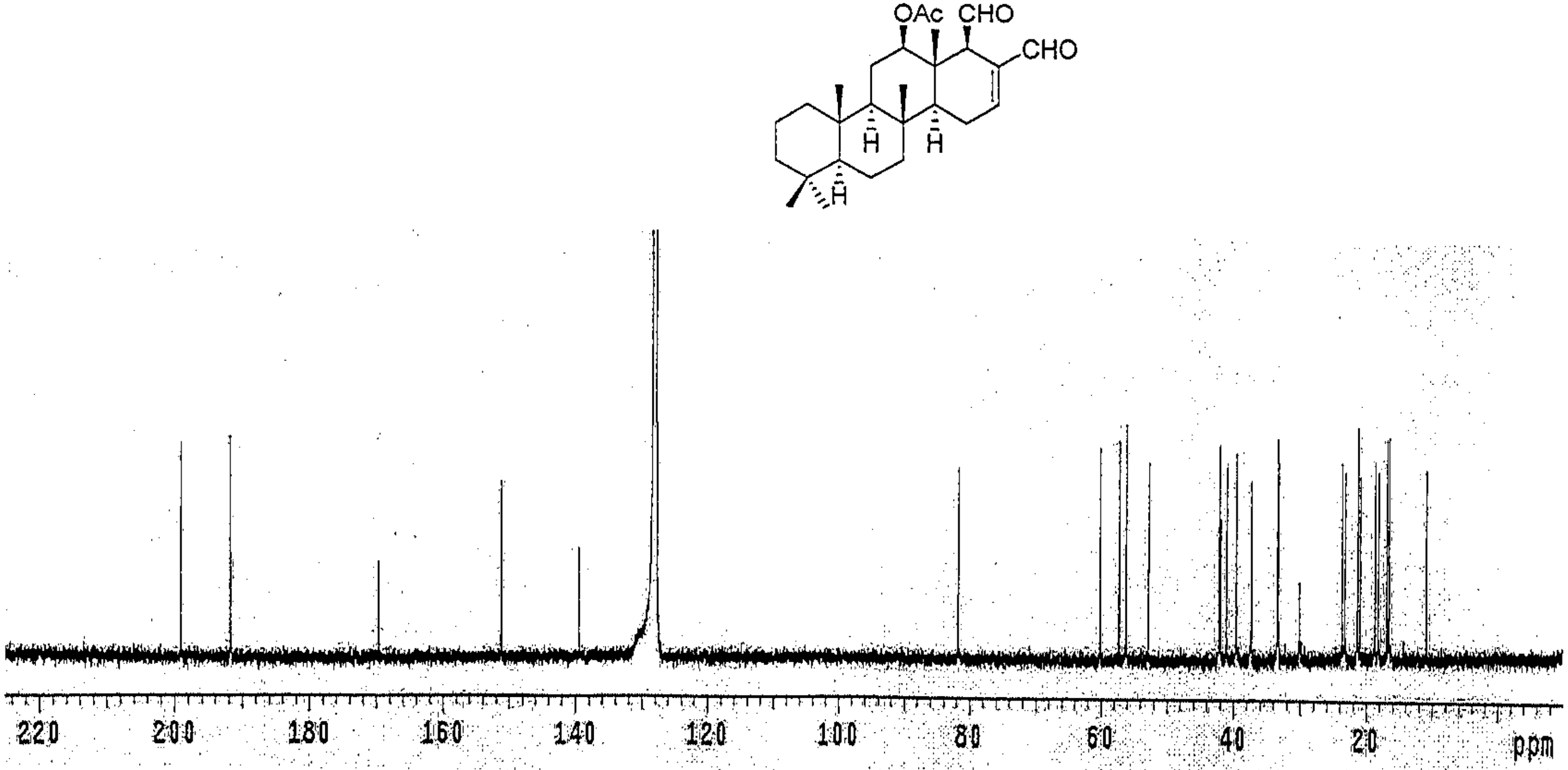
EI mass spectrum of compound 39



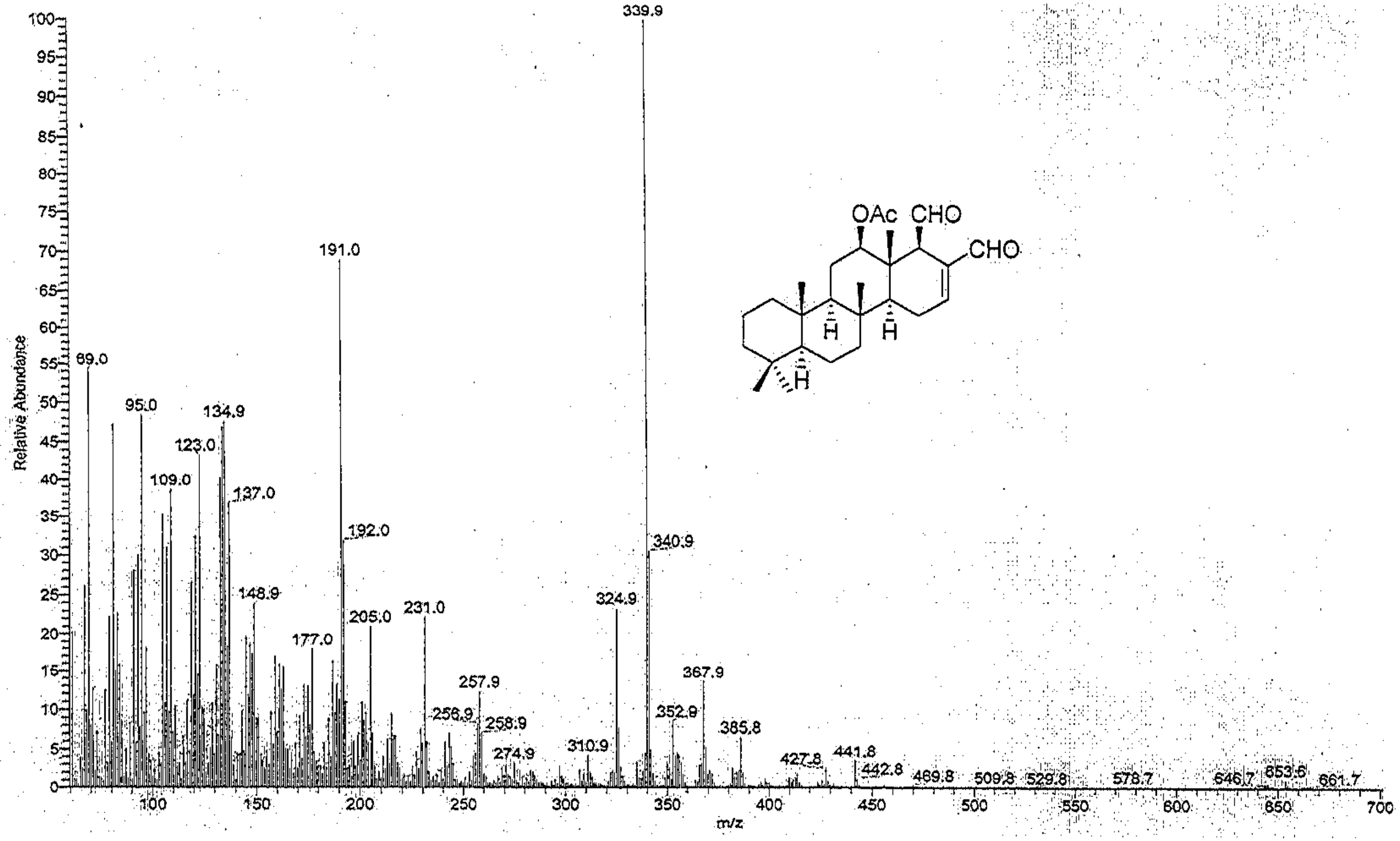
UV spectrum of compound 52 (MeOH)



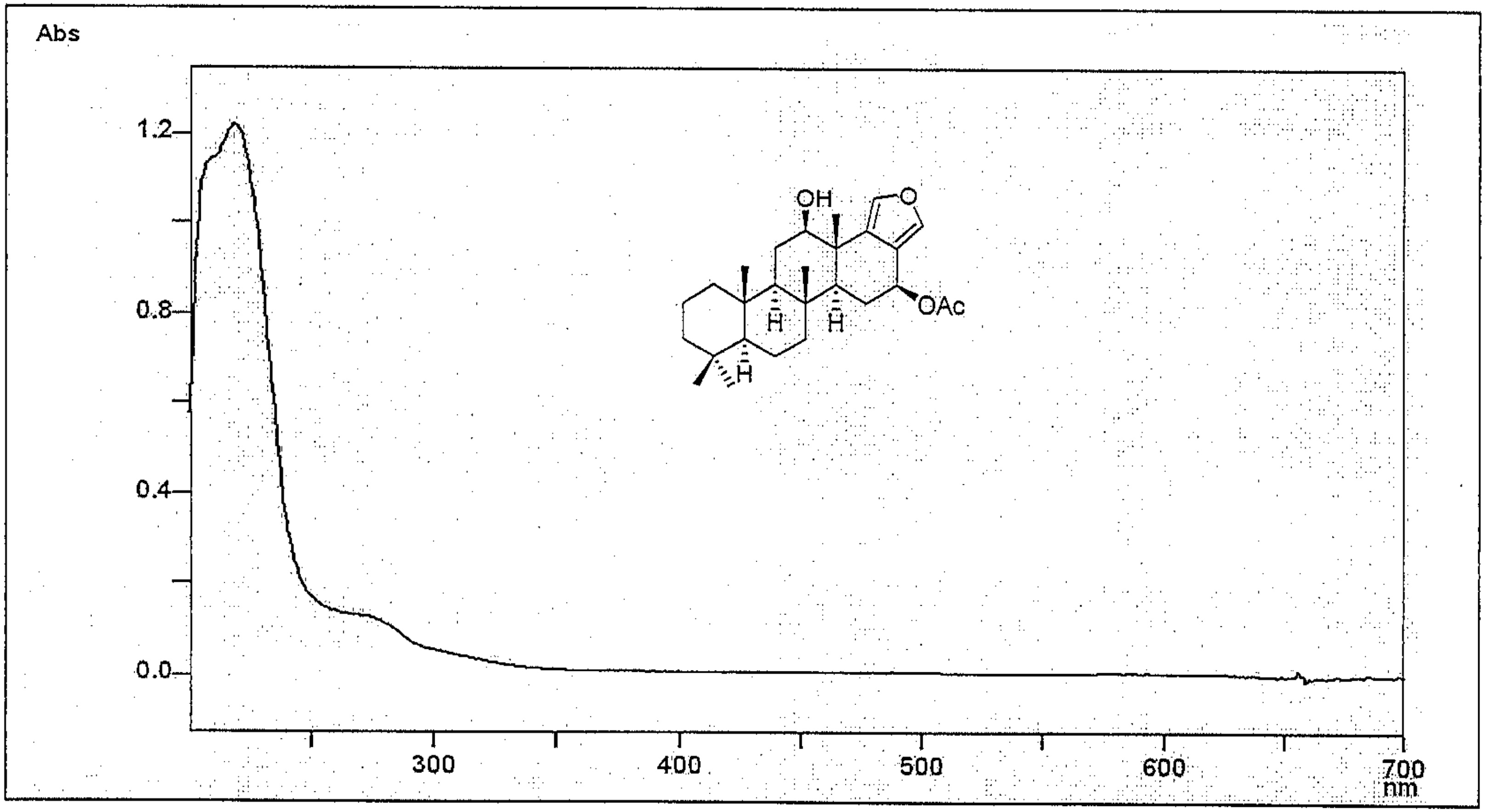
^{1}H NMR spectrum of compound 52 (500 MHz, C_6D_6)



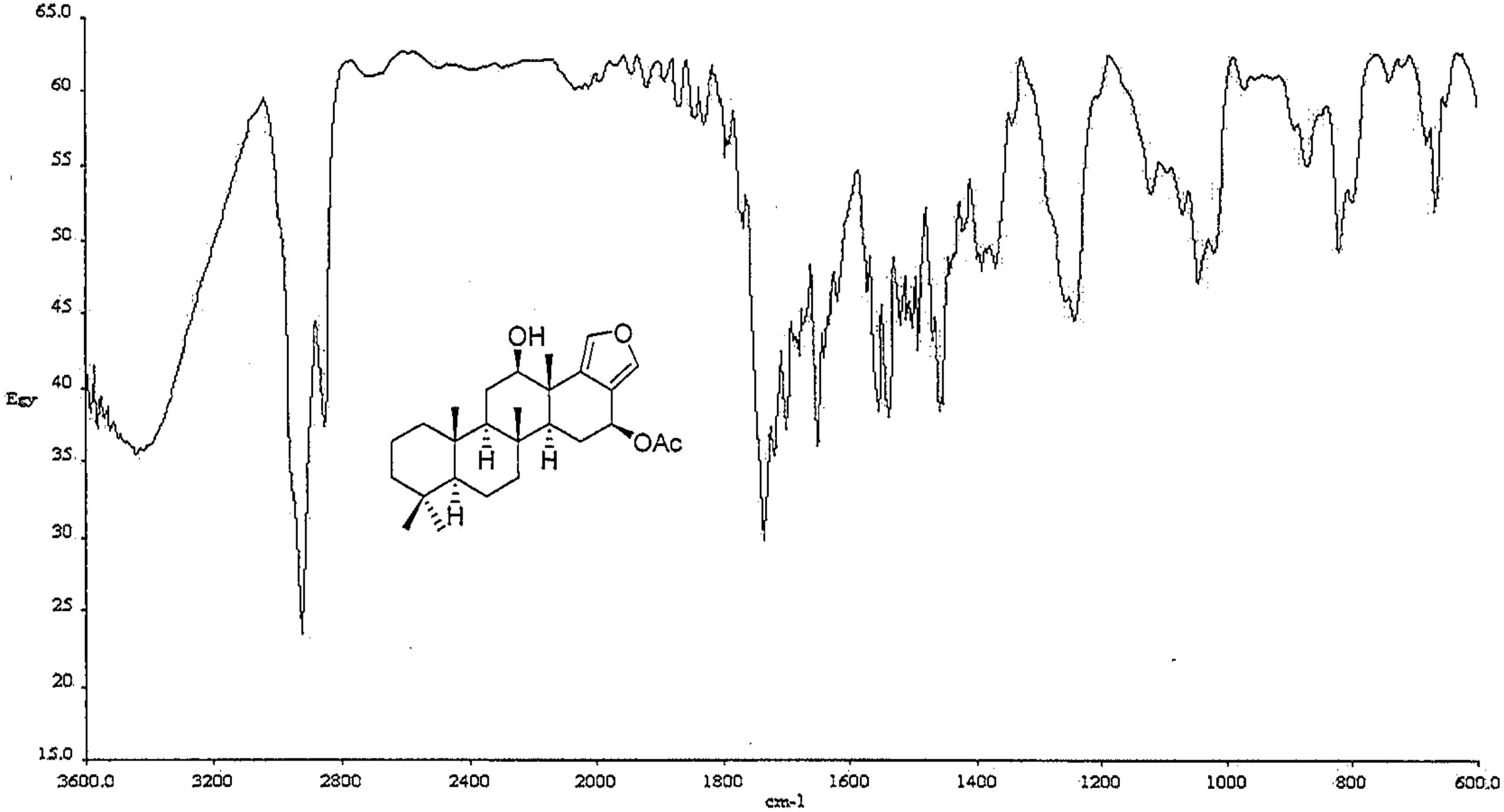
^{13}C NMR spectrum of compound 52 (125 MHz, C_6D_6)



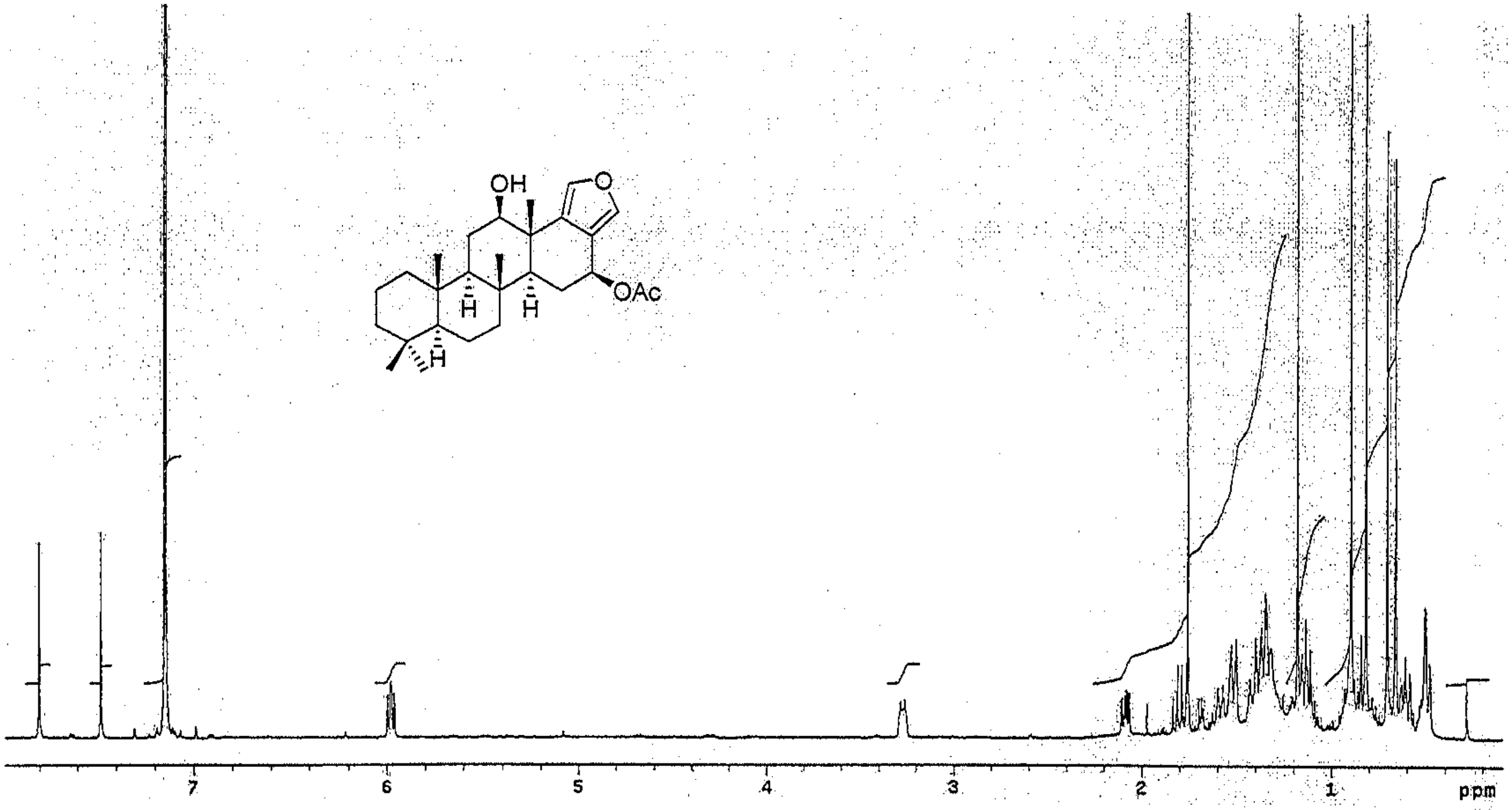
EI mass spectrum of compound 52



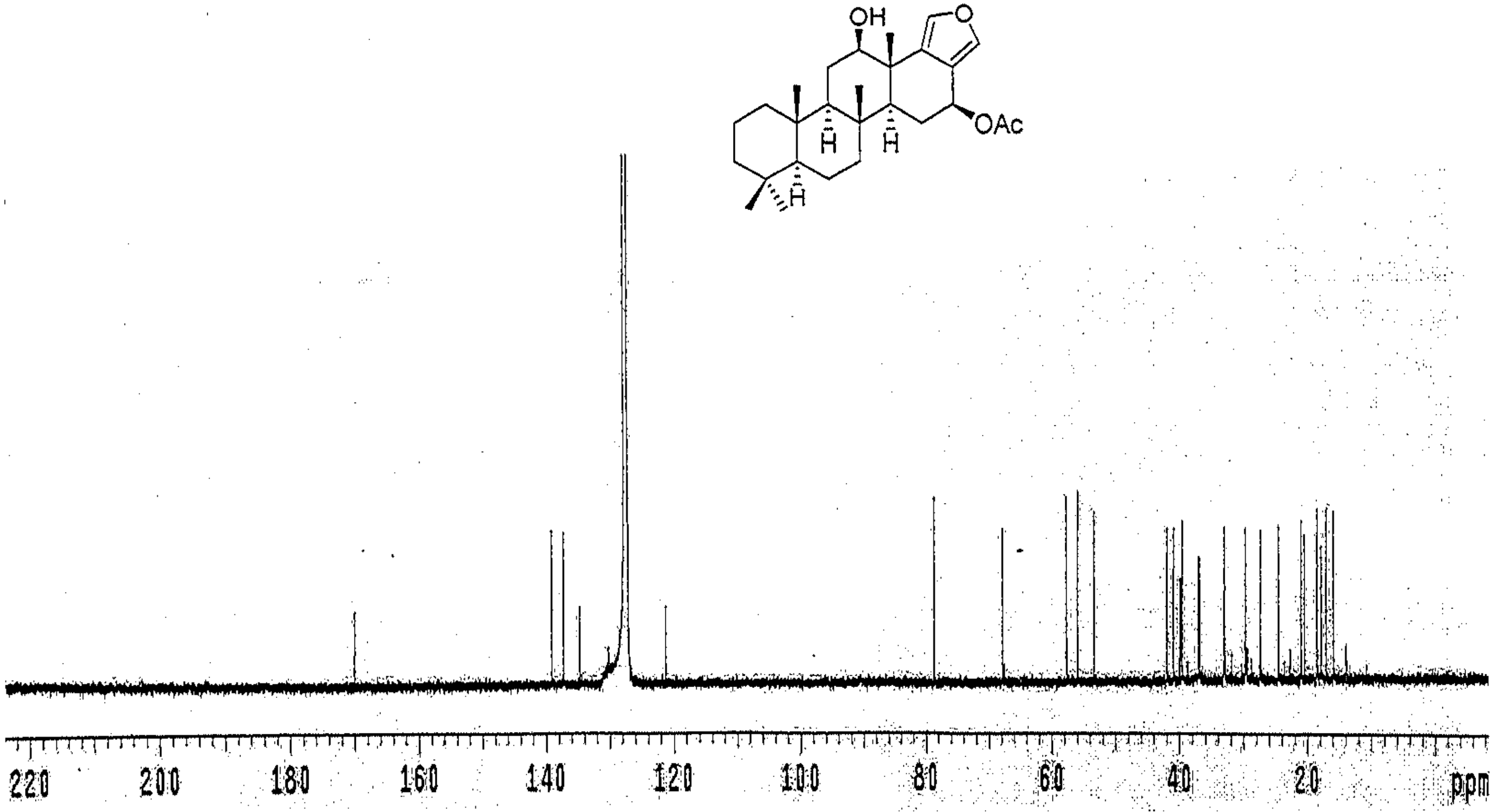
UV spectrum of compound 53 (MeOH)



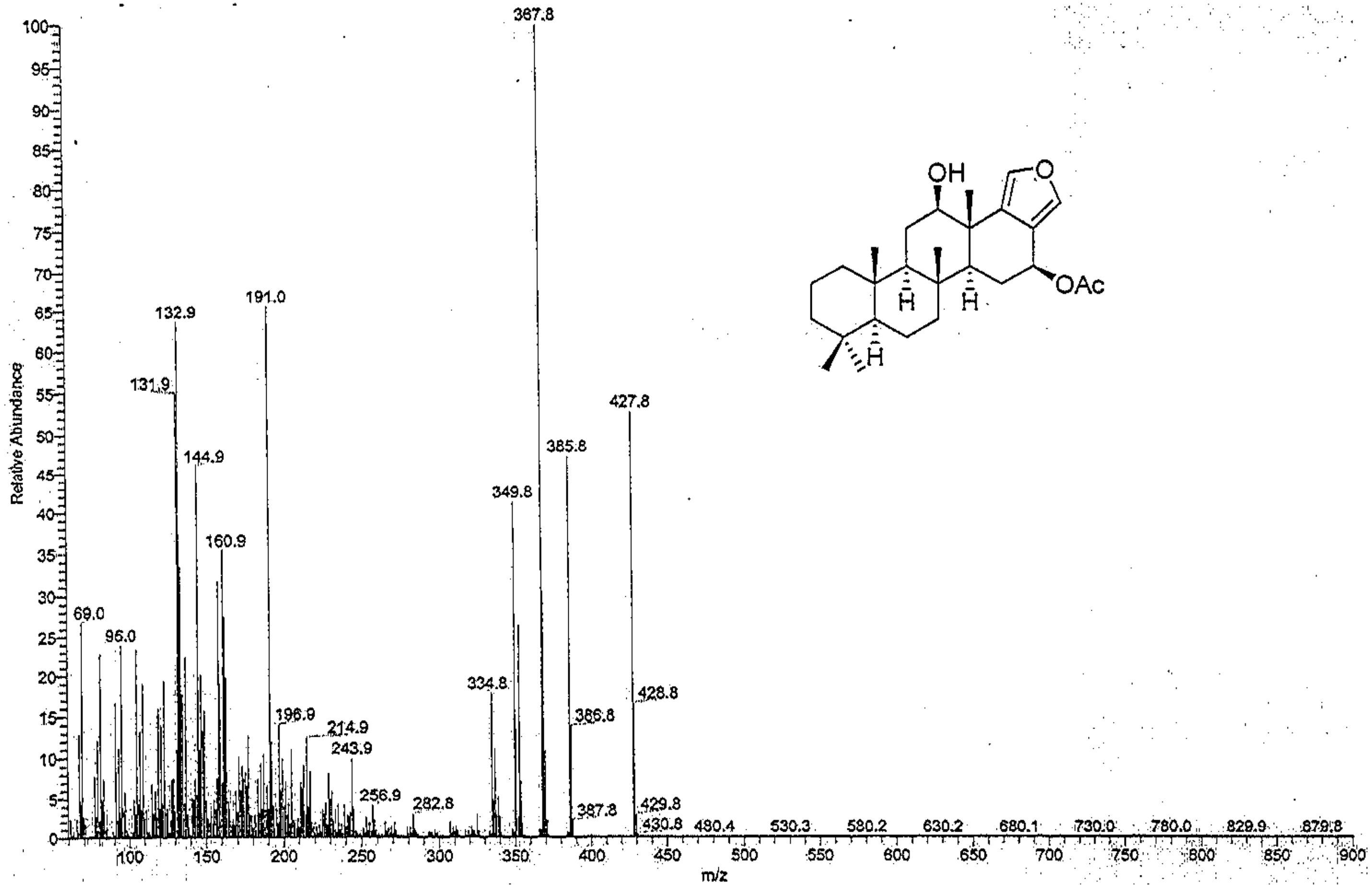
IR spectrum of compound 53 (neat)



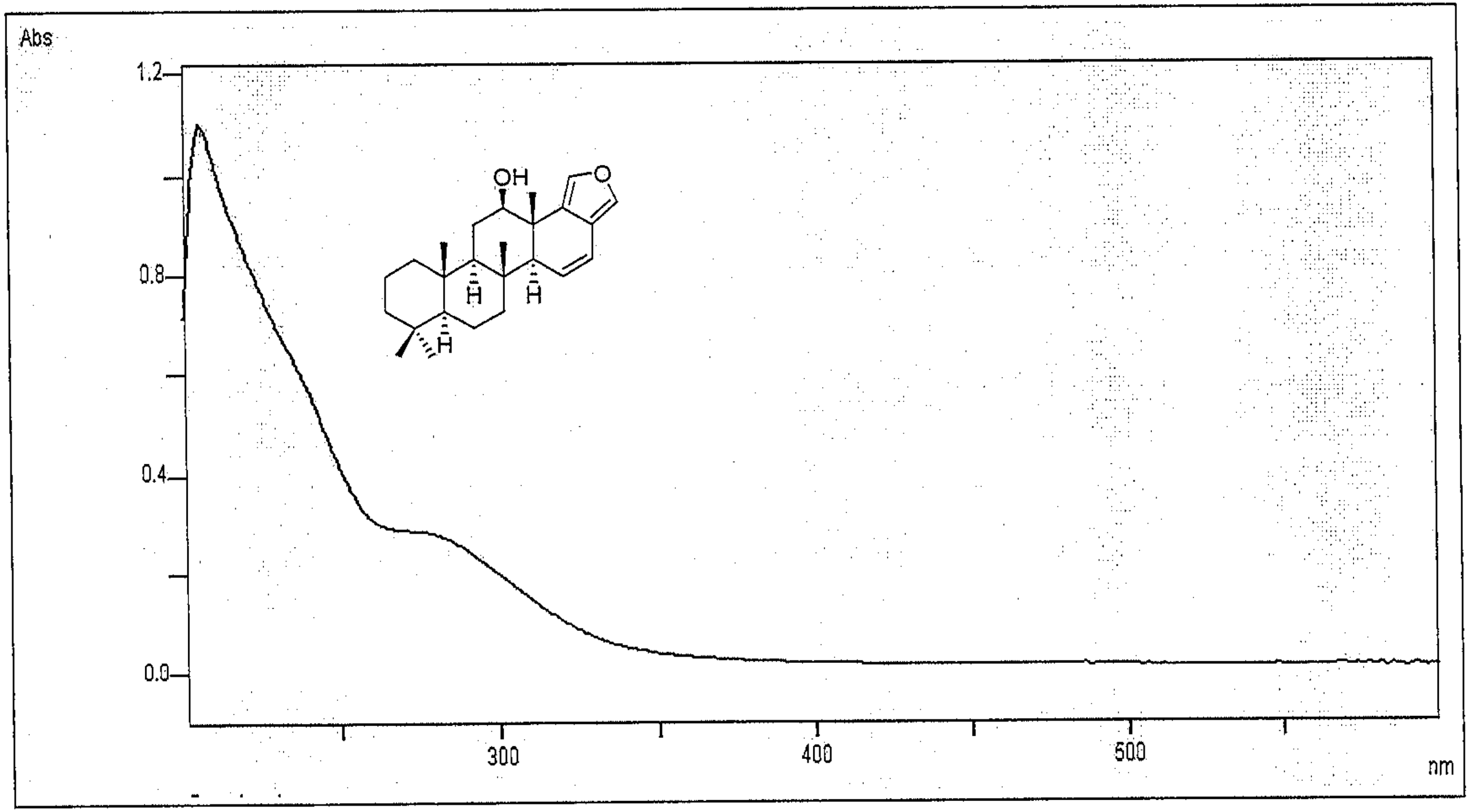
^{1}H NMR spectrum of compound 53 (500 MHz, C_6D_6)



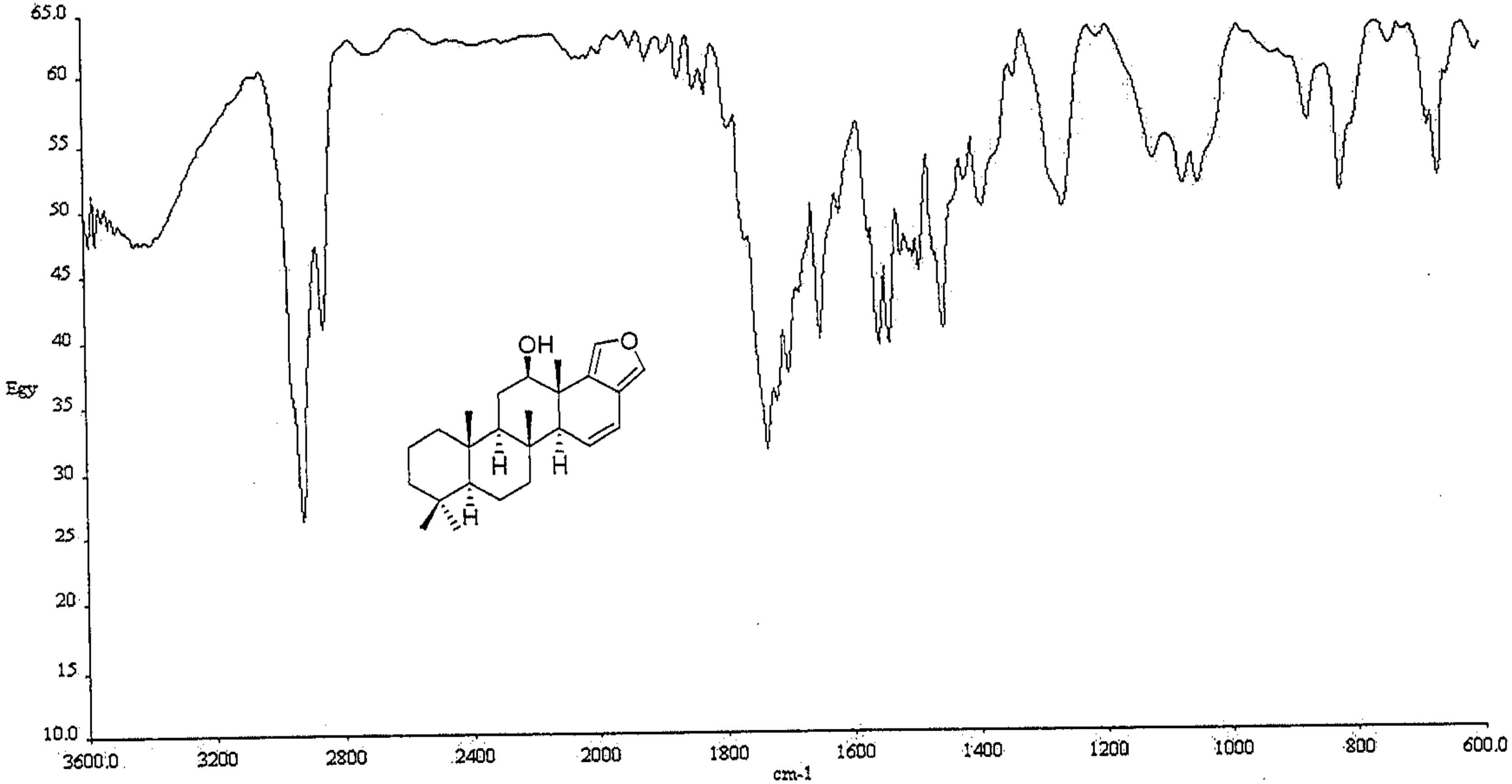
^{13}C NMR spectrum of compound 53 (125 MHz, C_6D_6)



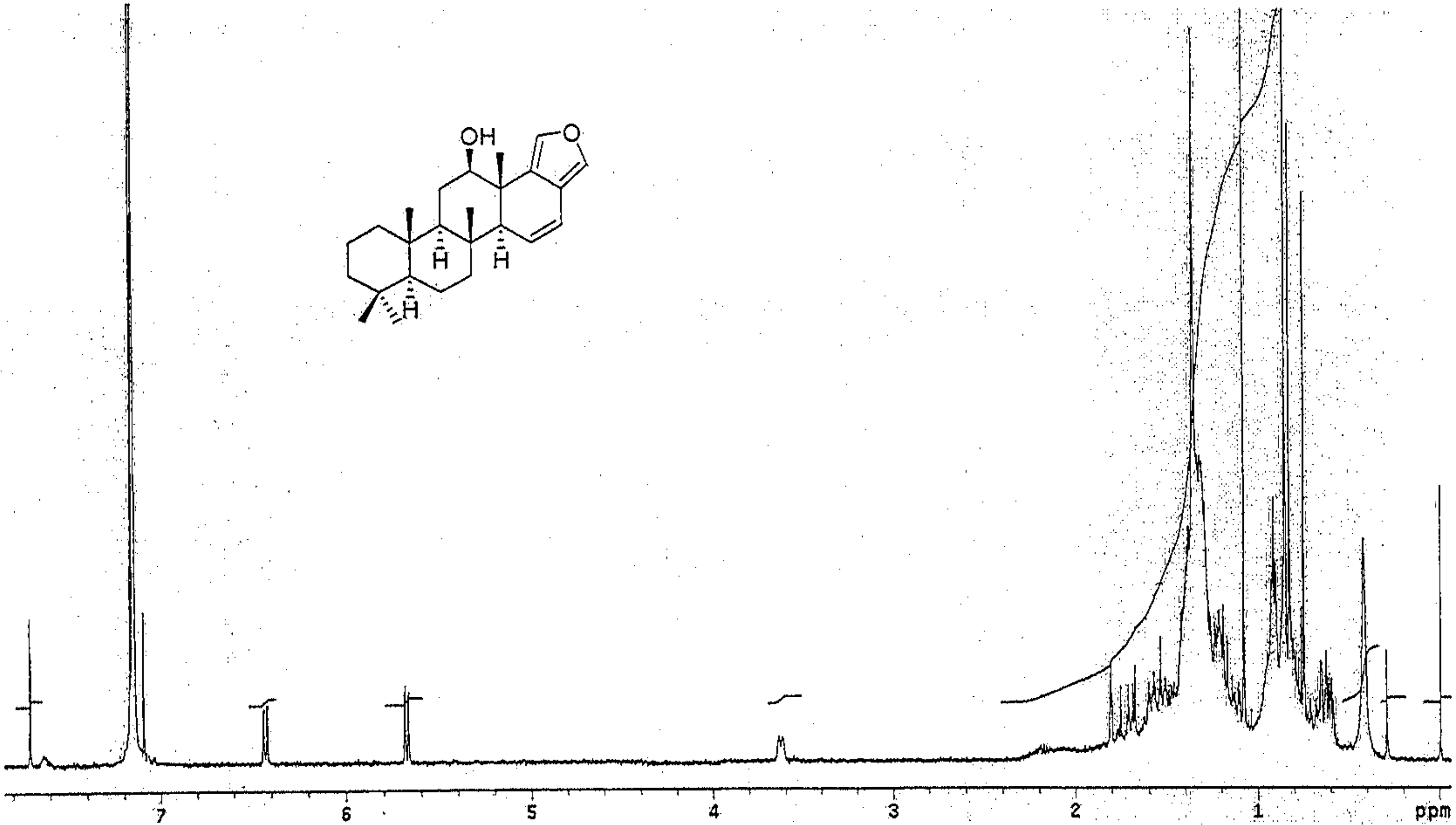
EI mass spectrum of compound 53



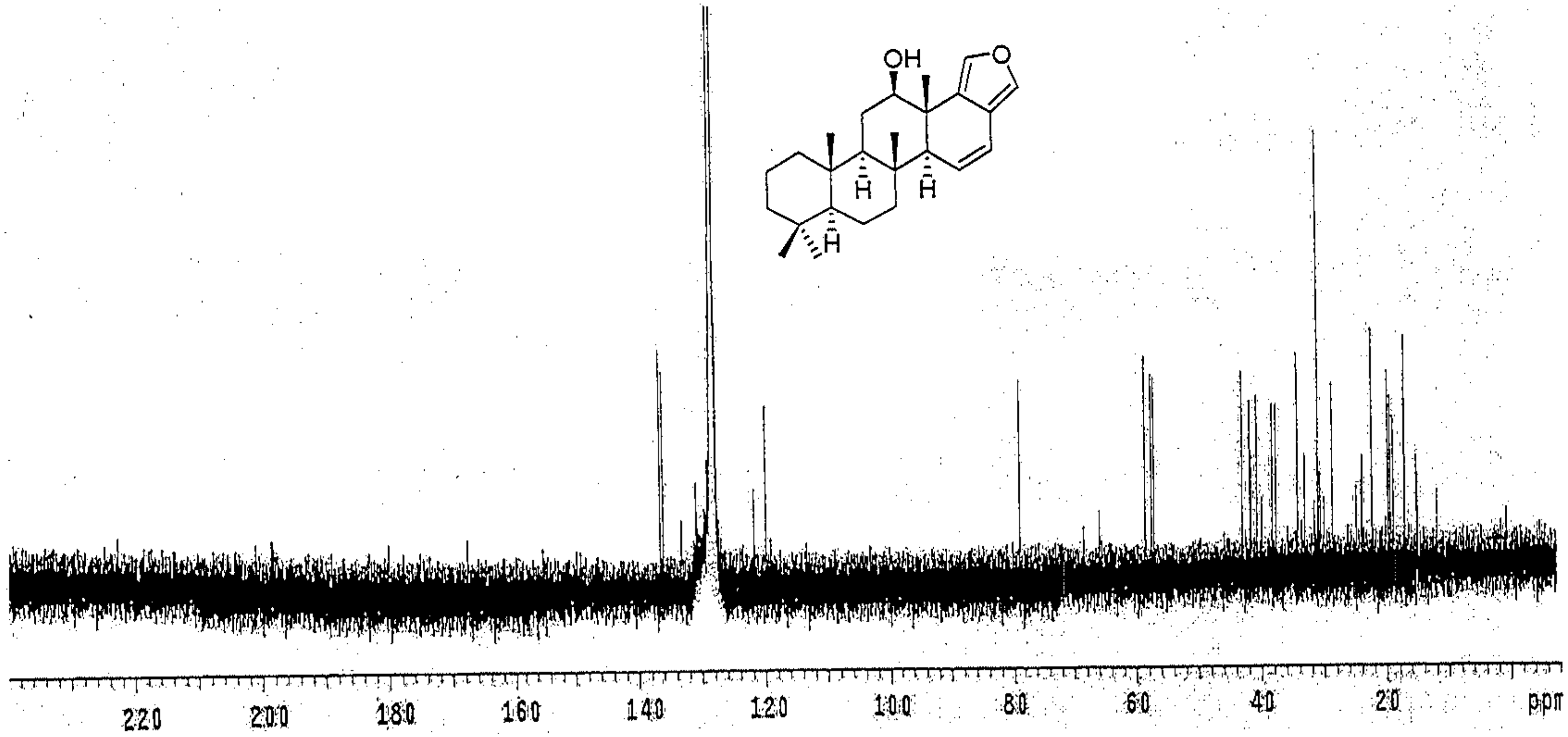
UV spectrum of compound **54** (MeOH)



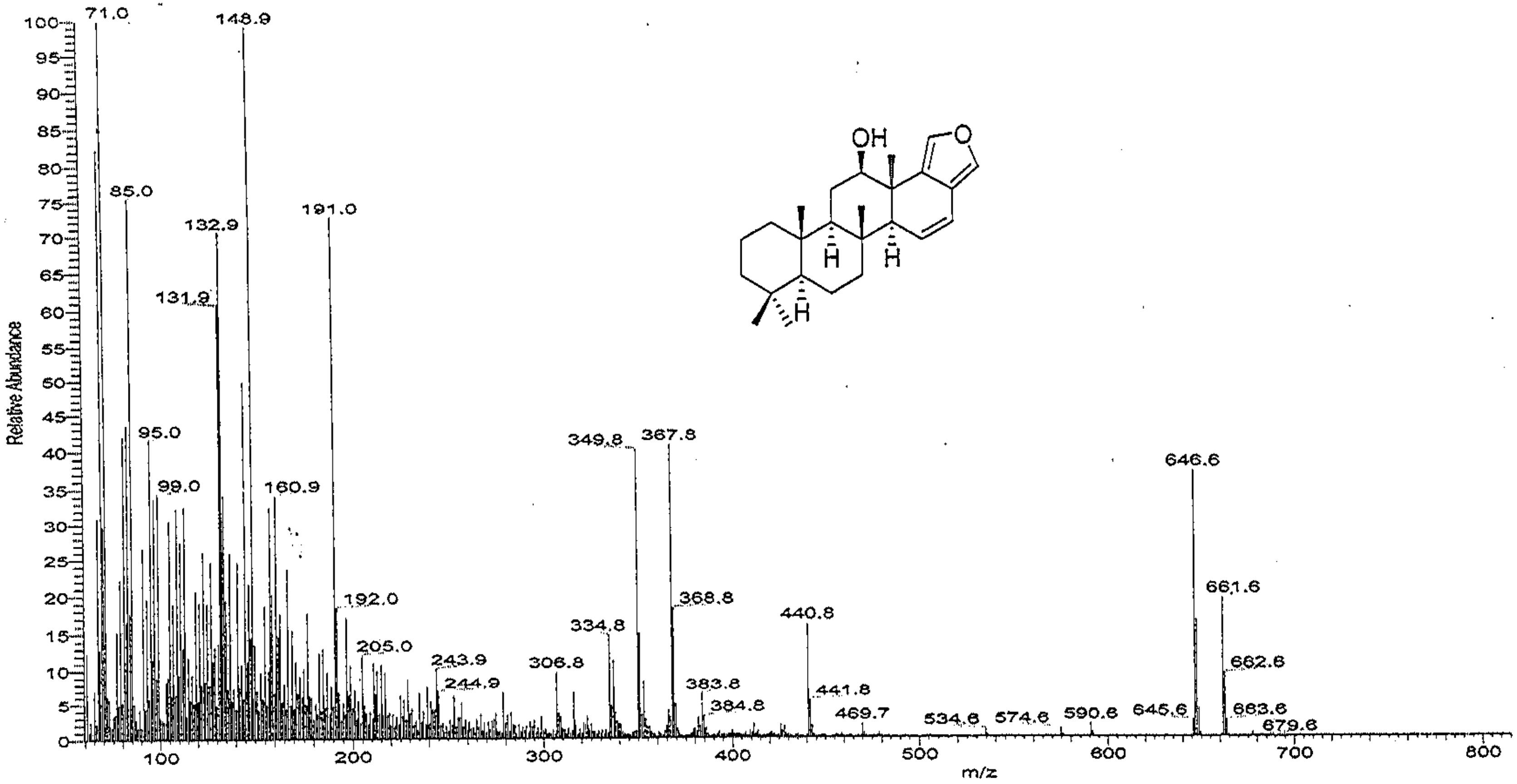
IR (neat) spectrum of compound 54



^{1}H NMR spectrum of compound **54** (500 MHz, C_6D_6)



^{13}C NMR spectrum of compound 54 (125 MHz, C_6D_6)



EI mass spectrum of compound 54

VITAE

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Education Attainment

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Bachelor of Science (Second Class Honors) (Chemistry)	Suratthani Rajabhat University	2004

List of Publication and Proceeding

Jaisamut, S., Karalai, C. and Plubrukarn, A. 2007. Antitubercular marine-derived sesterterpenoid derivatives. 2nd Sino-Thai international conference on traditional medicine and natural health products. Kosa Hotel, Khon Kaen, Thailand.