Antituberculosis Agents from Thai Sponge *Brachiaster* sp.

Saeng-ngam Wonganuchitmeta

Master of Pharmacy Thesis in Pharmaceutical Sciences

Prince of Songkla University

2003

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โครงการพัฒนาองค์ความรู้และศึกษานโยบายการจัดการทรัพยากรชีวภาพในประเทศไทย
c/o ศูนย์พันธุวิศวกรรมและเทคโนโลยีชีวภาพแห่งชาติ
อาคารสำนักงานพัฒนาวิทยาศาสตร์และเทคโนโลยีแห่งชาติ
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Author	Miss Saeng-ngam Wonganuchitmeta
Major program	Pharmaceutical Sciences
Academic Year	2003

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.....

(Associate Professor Dr.Surapon Arrykul)

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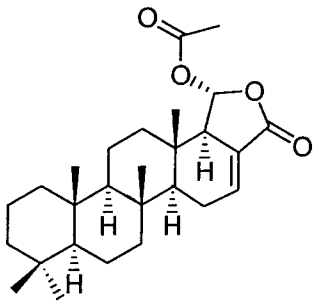
ชื่อวิทยานิพนธ์	สารที่มีฤทธิ์ต้านเชื้อวัณโรคจากฟองน้ำของไทยสกุล *Brachiaster*
ผู้เขียน	นางสาวแสงงาม วงษ์อนุชิตเมธา
สาขาวิชา	เภสัชศาสตร์
ปีการศึกษา	2546

บทคัดย่อ

การแยกสกัดสารควบคู่ไปกับการทดสอบฤทธิ์ต้านเชื้อวัณโรคจากสารสกัดจากฟองน้ำของไทยสกุล *Brachiaster* สามารถแยกสารประกอบกลุ่ม sesterterpenes ได้ 8 ชนิด โดยเป็นสารประกอบชนิดใหม่ 3 ชนิด ได้แก่ 12-deacetoxy-scalarin acetate (**40**), (*E*)-neomanoalide diacetate (**44**) และ (*Z*)-neomanoalide diacetate (**45**) และสารที่มีการรายงานโครงสร้างแล้ว 5 ชนิด ได้แก่ heteronemin (**18**), heteronemin acetate (**41**), 12-epi-19-deoxyscalarin (**42**), 12-deacetyl-12-epi-19-deoxyscalarin (**43**) และ manoalide-25-acetate (**46**) โดยวิเคราะห์หาสูตรโครงสร้างของสารเหล่านี้ใช้วิธีทางสเปคโตรสโคปี ยกเว้นสาร **18** และ **46** ซึ่งเคยมีรายงานฤทธิ์มาแล้ว รายงานฉบับนี้เป็นการรายงานฤทธิ์ต้านเชื้อวัณโรคและฤทธิ์ความเป็นพิษต่อเซลล์ครั้งแรกของสารที่สามารถแยกได้ทั้ง 8 ชนิด ทั้งนี้ พบว่าฤทธิ์ต้านเชื้อวัณโรคต่อเชื้อ *Mycobacterium tuberculosis* สายพันธุ์ $H_{37}Ra$ ของสารประกอบ **40**, **18**, **41** และ **46** มีความแรงในระดับ MIC 1.56, 1.56, 3.125 and 3.125 μg/mL ตามลำดับ และจากการทดสอบฤทธิ์ความเป็นพิษต่อเซลล์ พบว่าเฉพาะสารประกอบ **18** และ **46** แสดงฤทธิ์ความเป็นพิษต่อเซลล์มะเร็งทุกชนิดที่ IC_{50} น้อยกว่า 1 μg/mL



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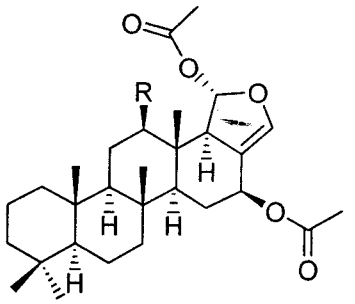

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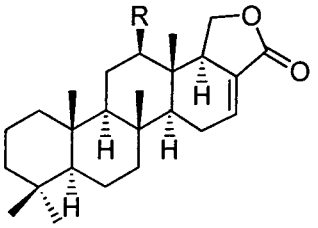

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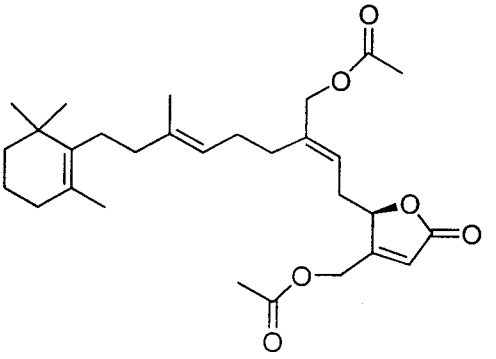

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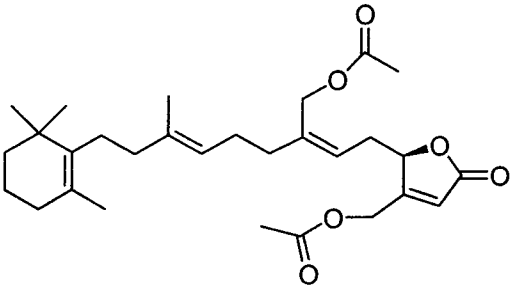

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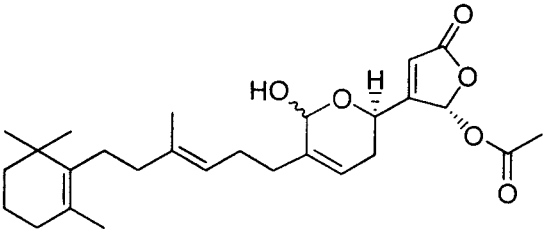

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Thesis Title	Antituberculosis Agents from Thai Sponge *Brachiaster* sp.
Author	Miss Saeng-ngam Wonganuchitmeta
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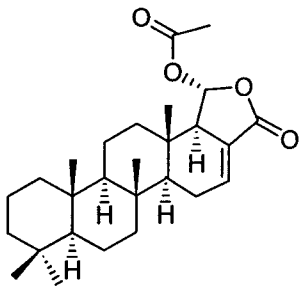
Abstract

The bioassay-guided fractionation of the Thai sponge *Brachiaster* sp. led to the isolation of eight sesterterpenes, including three new naturally-occurring compounds, 12-deacetoxy-scalarin acetate (**40**), (*E*)-neomanoalide diacetate (**44**) and (*Z*)-neomanoalide diacetate (**45**), along with five previously reported sesterterpenes, heteronemin (**18**), heteronemin acetate (**41**), 12-epi-19-deoxyscalarin (**42**), 12-deacetyl-12-epi-19-deoxyscalarin (**43**) and manoalide-25-acetate (**46**). The structure elucidation was achieved by means of spectroscopic analyses, particular NMR and CD spectroscopy. Excepted for compounds **18** and **46**, the antituberculosis and cytotoxic activities of all the isolated compounds were first reported here to show that compounds **40**, **18**, **41** and **46** exhibit potent antituberculosis activity against *Mycobacterium tuberculosis* strain $H_{37}Ra$ (MICs 1.56, 1.56, 3.125 and 3.125 μg/mL, respectively). On the other hand, the significant cytotoxicity was observed only in compounds **18** and **46**, with IC_{50} lower than 1 μg/mL.



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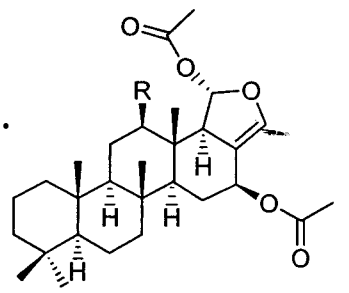

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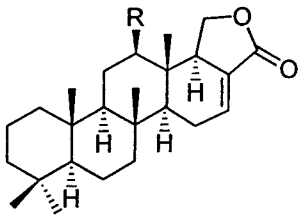

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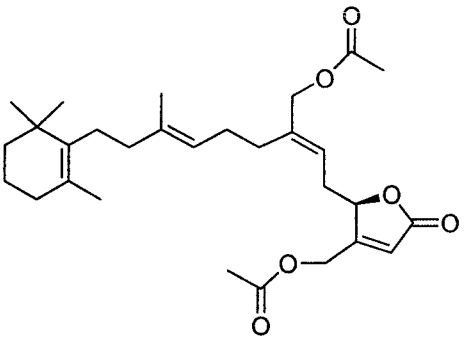

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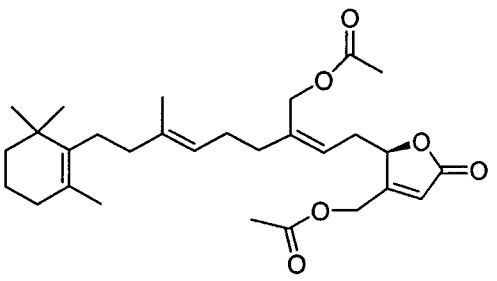

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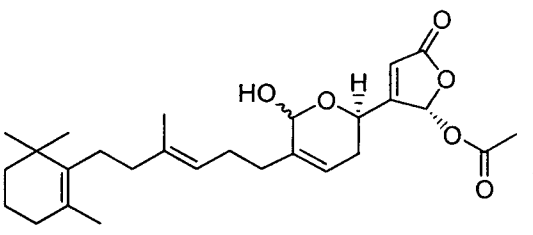

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Saeng-ngam Wonganuchitmeta

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ABBREVIATIONS AND SYMBOLS

λ_{max}	=	maximum wavelength
$[\alpha]_D$	=	specific rotation
δ	=	chemical shift (in ppm)
ε	=	molar extinction coefficiency
ν	=	wave number
Ac	=	acetyl
AIDS	=	acquired immune deficiency syndrome
ax	=	axial
BCG	=	Bacillus Calmette-Guerin vaccine
br	=	broad
c	=	concentration
CD	=	circular dichroism
CFU	=	colony forming units
COSY	=	correlation spectroscopy
d	=	doublet
DMSO	=	dimethylsulfoxide
EC_{50}	=	effective concentration at 50% of test subject
eq	=	equatorial
ESIMS	=	electrospray ionization mass spectroscopy
HIV	=	human immunodeficiency virus
HMBC	=	heteronuclear multiple-bond coherence

ABBREVIATIONS AND SYMBOLS (Cont.)

HMQC	=	heteronuclear multiple-quantum coherence
HPLC	=	high pressure liquid chromatograpy
HREIMS	=	high-resolution electron-impact mass spectroscopy
HRESIMS	=	high-resolution electrospray ionization mass spectroscopy
IC_{50}	=	inhibitory concentration at 50% of test subject
IR	=	infrared
J	=	coupling constant
LD_{50}	=	lethal dose at 50% of test sample
m	=	multiplet
m/z	=	mass over charge ratio
MABA	=	microplate alamar blue assay
MDR	=	multi-drug resistant
MIC	=	minimum inhibitory concentration
NMR	=	nuclear magnetic resonance
nOe	=	nuclear Overhauser effect
PLA_2	=	phospholipase A_2
s	=	singlet
SEM	=	standard error of mean
SRB	=	sulphorhodamine B
t	=	triplet
TB	=	tuberculosis

ABBREVIATIONS AND SYMBOLS (Cont.)

TCA	=	trichloroacetic acid
TLC	=	thin layer chromatography
T_R	=	retention time
UV	=	ultraviolet-visible

CHAPTER 1

INTRODUCTION

1.1 General introduction

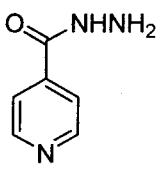
Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, is currently considered among the most dangerous infectious diseases world-wide, and is one of the major AIDS-associated infections (Inderlied, 1999). According to the alarming data furnished by the World Health Organization (WHO), one-third of the world population is infected with *M. tuberculosis*, and there are approximately eight million new cases and more than two million deaths reported each year. In particular, three of the four highest-burden countries are in Southeast Asia. Besides that, Thailand is ranked among the top 22 TB-high burden countries, with 88,000 new cases in the year 2000 (Dye *et al.*, 1999; WHO, 2002).

Despite the availability of a vaccine (BCG) and effective chemotherapeutic agents against TB since 50 years ago, TB was ironically declared a global emergency in 1993 (Crofton, 1997). The prime factors contributing to such declaration are due to a high prevalence of TB in patients who have AIDS and to multi-drug resistant strains of mycobacteria, thus causing the number of patients infected with TB to increase world-wide (Glassroth, 2001).

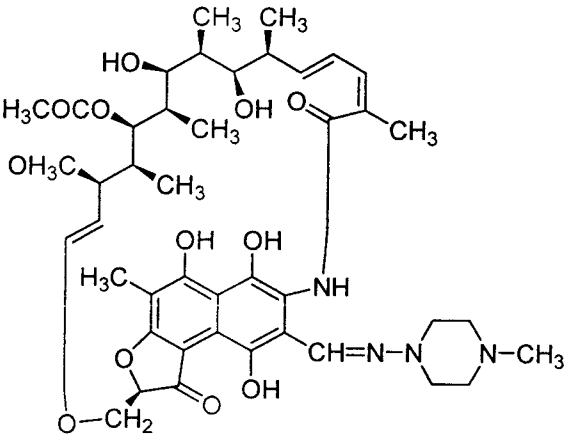
HIV infection has increased the incidence of TB by causing immunosuppression, which enables latent infection to clinically progress (Glassroth, 2001). There are approximately 10.7 million people having TB/HIV coinfection (0.18% of the world population), and 640,000 cases were associated with HIV infection (Dye *et al.*, 1999). Unlike other diseases associated with AIDS, the severe uniqueness of TB is that it can be spread by airborne transmission to adults and children who are not at risk of AIDS (Haas and Des Prez, 1995).

Resistance to the current antituberculosis drugs is another threatening problem. First-line drugs currently used in the treatment of TB include isoniazid, rifampin, 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. The major problems faced in TB control are poverty, thus leading to the lack of diagnosis and short in drug supply, and patients' 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 rifampin, have been emerged (Duncan, 1997; Inderlied, 1999). There were approximately 3.2% of newly estimated TB cases world-wide 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

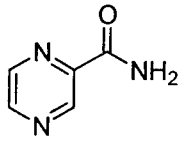
and/or more toxic than first-line ones, are obligated in such cases (Glassroth, 2001).



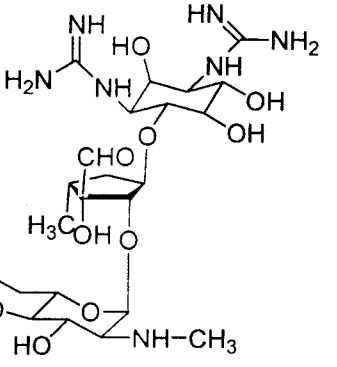
isoniazid



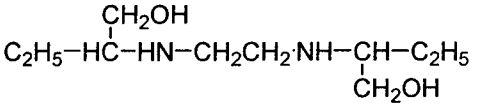
rifampin



pyrazinamide



streptomycin



ethambutol

Figure 1 First-line drugs for tuberculosis

In spite of the advance in computer-assisted drug design, molecular biology and gene therapy, there is still a pressing need for new drugs to counteract with multi-drug resistant tuberculosis. However, for over 30 years no antituberculosis agents with new mechanism of action 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 disease in the developing world. The very slow growth and highly contagious nature of *M. tuberculosis* have also discouraged the drug discovery effort (Cantrell, Franzblau and Fischer, 2001). Yet new drug discovery with new and different mode 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.

Nature is one attractive source of new therapeutic candidates as the tremendous chemical diversity is found in millions of species of both marine and terrestrial plants, animals and microorganisms. Despite major scientific and technological progresses in combinatorial chemistry, drugs derived from natural products, however, still make an enormous contribution to drug discovery today. Of the new approved drugs reported between 1983 and 1994, for examples, drugs of natural origins predominate (78%) in the area of antibacterials, whereas 61% of anticancer drugs are naturally-derived or are modeled on natural product parents (Cragg, Newman and Snader, 1996).

The oceans, covering more than 70% of the earth's surface, have been long known as the ecological habitat with a highly unique and wide-ranged biodiversity. Such uniqueness that earns marine biota the excellence candidacy as the producers of novel biologically active agents include the physical and chemical differences between the marine and terrestrial environments. Among these differences are the great density of the sea water, the reduced light permeation thus allowing

photosynthesis only in a narrow surface zone, and the skeleton of the biosynthetically 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. These properties result in the abundance of filter-feeding sessile organisms, which serve as excellent substrata for epibionts and symbionts, therefore becoming the communities that are either absent or rare in terrestrial ecosystems (Scheuer, 1990). Furthermore, ecological stresses, including predation, competition for space, and fouling of the surface, lead to the evolution of unique secondary metabolites with various biological activities (Konig *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.

Since the first reports in 1951, marine plants, animals and microbes have already yielded more than 12,000 novel chemicals, with hundreds of new compounds still being discovered every year (Donia and Hamann, 2003). The isolation of two new unusual arabinonucleosides, spongothymidine and spongouridine from the sponge *Cryptotethia crypta* by Bergmann in 1950's led to the development of several nucleoside analogues, including ara-C as anticancer agent, and acyclovir as antiviral drug for *Herpes simplex* virus infections (Munro *et al.*, 1994). Currently, ara-C and acyclovir are the only marine-related compounds in clinical use. However, many marine natural products and their derivatives have successfully advanced to the stages of clinical trials, especially in the area of

chemotherapy (Table 1) (Munro *et al.*, 1999; Haefner, 2003). Additionally, the reviews by Mayer and Hamann (2002) reported a growing number of candidates that have been selected as promising leads for extended preclinical assessment.

Whereas most of natural products in clinical trials are aimed toward anticancer chemotherapy, the emerging drug resistance encountered in the infectious diseases also contributes to the interest in assessing marine natural products. There are many marine natural products that have been described for their potent antiinfective activities and show their potential toward clinically useful treatments (Mayer and Hamann, 2002; Donia and Hamann, 2003).

Among the marine organisms, sponges were the first marine invertebrate group that have been studied in search for new compounds (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, the sponges have had 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-

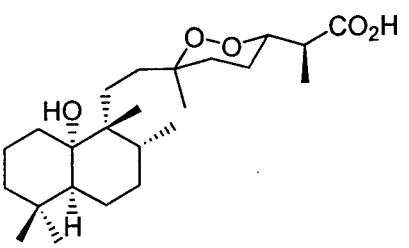
Table 1 Marine natural products and derivatives in clinical development

Compound	Source	Chemical class	Disease area	Status
Compounds targeting ion channels				
Ziconotide	Cone snail	Peptide	Chronic pain	Phase III
AM336	Cone snail	Peptide	Chronic pain	Phase I/II
GTS21	Nemertine worm	Anabaseine-derivative	Alzheimer's disease Schizophrenia	Phase I/II
Compounds targeting enzymes				
Methionine aminopeptidase inhibitors				
LAF389	Sponge	Amino acid derivative	Cancer	Phase I
Protein kinase inhibitors				
Bryostatin-1	Bryozoan	Polyketide	Cancer	Phase II
PLA_2 inhibitors				
OAS1000	Soft coral	Diterpene-pentoseglycoside	Wound healing Inflammation	Phase I/II
Microtubule-interfering agents				
Dolastatin-10	Sea slug	Peptide	Cancer	Phase II
ILX651	Sea slug	Peptide	Cancer	Phase I
Cemadotin	Sea slug	Peptide	Cancer	Phase II
Discodermolide	Sponge	Polyketide	Cancer	Phase I
HTI286	Sponge	Tripeptide	Cancer	Phase I
DNA-interactive agents				
Yondelis™	Sea squirt	Isoquinolone	Cancer	Phase II/III
Oxidative stress inducers				
Aplidin™	Sea squirt	Cyclic depsipeptide	Cancer	Phase II
Lysosomotropic compounds				
Kahalalide F	Sea slug/alga	Cyclic depsipeptide	Cancer	Phase I
Immunostimulatory agents				
KRN7000	Sponge	β-galactosylceramide	Cancer	Phase I
Calcium-binding protein antagonists				
Squalamine lactate	Shark	Aminosteroid	Cancer	Phase II
Compounds with unknown mechanism of action				
IPL512602	Sponge	Steroid	Inflammation Asthma	Phase II

note; produced after Haefner (2003).

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 and symbionts 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 (Konig and Wright, 1996). It is thus not surprising that many marine natural products from sponges are highly active in many pharmacological assays.

Although Thailand's territorial waters, covering approximately more than 400,000 km^2, are one of the world's greatest biological diversified marine habitats (Allen, 1996), the researches in marine natural products are yet rather new to the Thai researchers. To date, there have been only a handful studies about the bioactive compounds from Thai marine organisms. For instances, Tanaka *et al.* (1993) reported the isolation of two new norsesterterpene peroxides, mycaperoxides A **(1)** and B **(2)**, from the Thai sponge *Mycale* sp. collected from Sichang Island, Chonburi. Both compounds exhibited cytotoxicity against P-388, A-549 and HT-29 tumor cell lines (IC_{50} 0.5-1 μg/mL).



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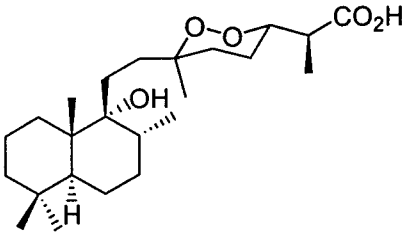

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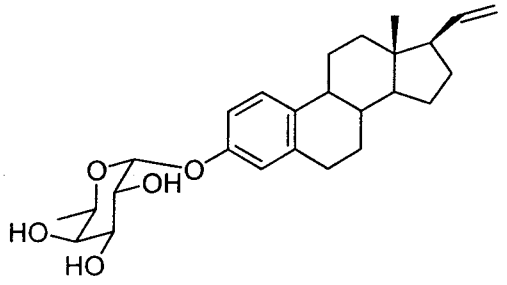
Kittakoop *et al.* (1999) reported the isolation of two new norpregnane glycosides, 19-norpregna-1,3,5(10),20-tetraen-3-*O*-α-fucopyranoside **(3)** and 19-norpregna-1,3,5(10),20-tetraen-3-*O*-β-arabinopyranoside **(4)**, from the Thai soft coral *Scleronephthya pallida* collected from Phuket. 19-Norpregna-1,3,5(10),20-tetraen-3-*O*-α-fucopyranoside exhibited moderate antimalarial (EC_{50} against *Plasmodium falciparum* 1.5 μg/mL) and cytotoxic (EC_{50} against BCA-1 breast cancer, 10 μg/mL) activities.



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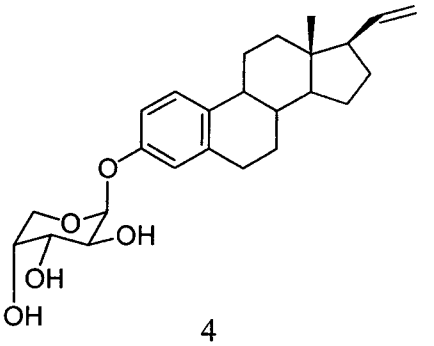
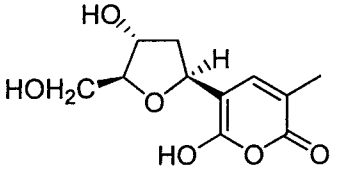


4

Watanadilok *et al.* (2001) reported the isolation of two unusual hydroxypyran-2-ones, tetillapyrone **(5)** and nortetillapyrone **(6)**, from the Thai sponge *Tetilla japonica* collected from Captain Yuth beach, Chonburi.



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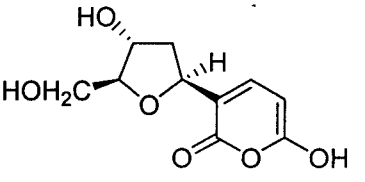
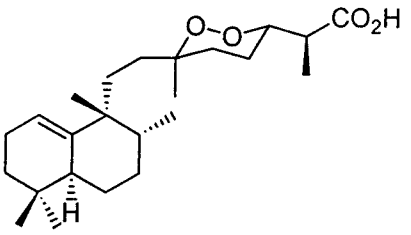


6

Most recently Phuwapraisirisan *et al.* (2003) reported the isolation of a new norsesterterpene peroxide, mycaperoxide H **(7)** from the Thai sponge *Mycale* sp.

collected from Sichang Island, Chonburi. This compound showed cytotoxic activity (IC_{50} 0.8 μg/mL against HeLa cells).



7

In our pilot study, we found that the extracts from several sponges collected from Koh-Tao, Suratthani, showed various potent biological activities including antimicrobial, cytotoxic and antituberculosis. Among these, the methanolic extract from a brown sponge, later identified as *Brachiaster* sp., exhibited potent anti-tuberculosis activity (MIC 12.5 μg/mL). These results along with the increasing prevalence and drug resistance of tuberculosis led to the initiation of a research project in search of new antituberculosis agents. The main objectives of this investigation are as the followings;

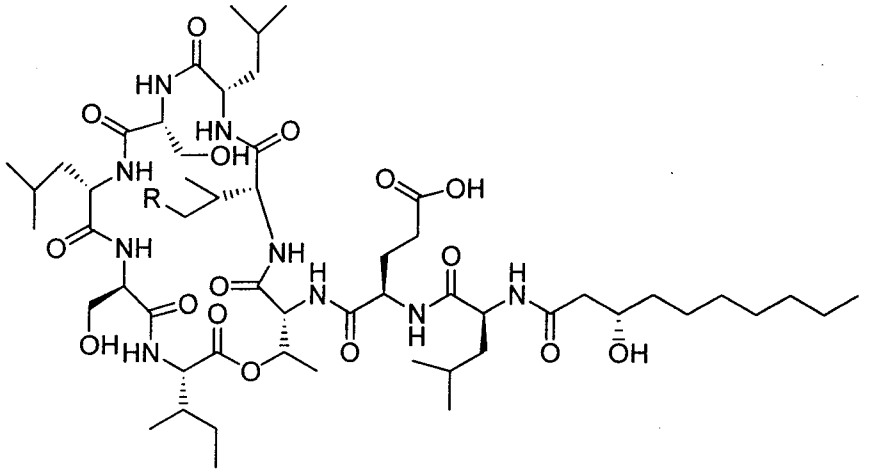
(i) to isolate antituberculosis constituents from the Thai sponge *Brachiaster* sp.,

(ii) to identify and elucidate the chemical structures of the isolated compounds, and

(iii) to propose the basic structure-activity relationship of the isolated compounds.

1.2 Marine natural products as antituberculosis agents

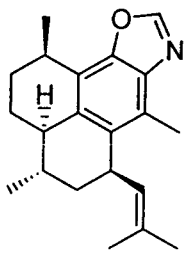
Whereas a large number of antimycobacterial agents from plant species were reported (for example, see review by Newton, Lau and Wright, 2000), to date there are a few reports regarding compounds with *in vitro* antituberculosis actitvity from the marine origins. The first report was the isolation of two cyclic depsipeptides, massetolide A **(8)** and viscosin **(9)**, from the cultures of two *Pseudomonas* species isolated from a marine alga and a tube worm, respectively. The two compounds exhibited antituberculosis activity against *M. tuberculosis* with MICs of 5-10 and 10-20 μg/mL, respectively (Gerard *et al.*, 1997).



8 : R = CH_3 9 : R = H

Pseudopteroxazole **(10)** and seco-pseudopteroxazole **(11)**, the benzoxazole diterpene alkaloids isolated from the West Indian gorgonian *Pseudopterogorgia elisabethae*, respectively induced 97 and 66% growth inhibition in *M. tuberculosis* $H_{37}Rv$ at a concentration of 12.5 μg/mL without substantial toxic effect. (Rodriguez *et al.*, 1999). Additionally, erogorgiaene **(12)**, a serrulatane diterpene

isolated from the same West Indian gorgonian, induced 96% growth inhibition in *M. tuberculosis* $H_{37}Rv$ at a concentration of 12.5 μg/mL (Rodriguez and Ramirez, 2001). It was proposed that the benzoxazole moiety is not essential for antituberculosis activity, as demonstrated by erogorgiaene.



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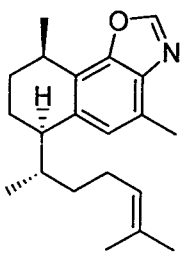

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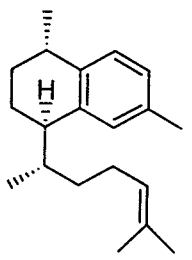
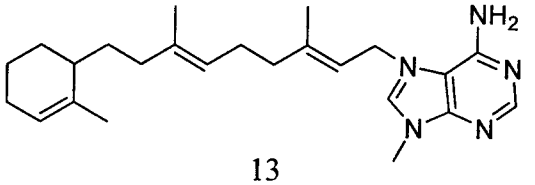


12

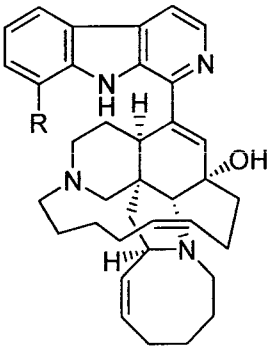
Agelasine F **(13)**, a monocyclic diterpenoid with a 9-methyladeninum unit isolated from the Philippine sponge *Agelas* sp. inhibited some drug-resistant strains of *M. tuberculosis* with MIC of 3.13 μg/mL (Mangalindan *et al.*, 2000).



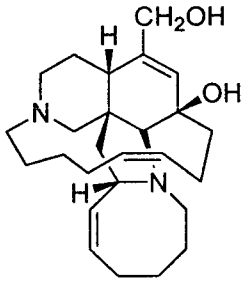
13

Manzamine A **(14)** and (+)-8-hydroxy-manzamine A **(15)**, two members of the unique β-carboline alkaloids, exhibited potent antituberculosis activity against *M. tuberculosis* $H_{37}Rv$ (MIC 1.53 and 0.91 μg/mL, respectively) (Yousaf *et al.*, 2002). These alkaloids were first isolated from sponge *Haliclona* sp. (Sakai and Higa, 1986) and *Pachypellina* sp. (Ichiba, Corgiat and Scheuer, 1994). Its presumed biogenetic precursor, ircinol A **(16)**, which does not possess the β-

carboline moiety, also exhibit the same activity at an MIC of 1.93 μg/mL. Ircinol A represents a useful candidate for *in vivo* assessment toward *M. tuberculosis* treatment, since it shows lower cytotoxicity and less structural complexity than other manzamine-type alkaloids (Yousaf *et al.*, 2002; Donia and Hamann, 2003).



14 ; R = H
15 ; R = OH

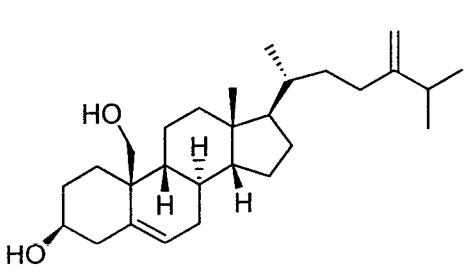


16

El Sayed *et al.* (2000) reported the promising antituberculosis activity of three compounds (90-99% inhibition of the growth of *M. tuberculosis*). The first one, litosterol **(17)**, C19-hydroxy steroids first isolated from the Okinawan soft coral *Litophyton virdis* (Iguchi, Saitoh and Yamada, 1989), inhibited 90% of the growth of *M. tuberculosis* $H_{37}Rv$ with an MIC of 3.13 μg/mL. It was reported that the poor solubility of litosterol in the aqueous culture media obscured the assessment of cytotoxic effects.

Heteronemin **(18)**, a scalarane-type sesterterpene primarily isolated from the sponge *Heteronema erecta* (Kazlauskas *et al.*, 1976), induced 99% inhibition with an MIC of 6.25 μg/mL and IC_{50} of 1.3 μg/mL. The high cytotoxicity of this

compound prohibited further biological evaluation; however, chemical modifications of this compound were suggested to produce less toxic and more active derivatives.



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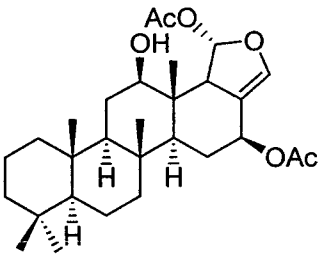
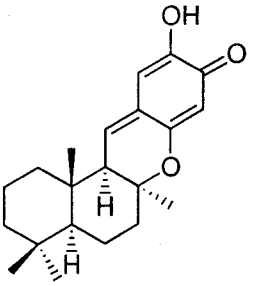


18

The last one, puupehenone **(19)**, was reported to induce 99% inhibition with an MIC of 12.5 μg/mL and IC_{50} of 2.0 μg/mL. The puupehenones are shikimate-sesquiterpene derived metabolites isolated from sponges of the order Verongida and Dictyoceratida from the Hawaiian Island (Nasu *et al.*, 1995).

In a report by Konig, Wright and Franzblau (2000), several compounds were subjected to antituberculosis activity determinations. It was found that the compound with the highest potency was axisonitrile-3 **(20)**, a cyanosesquiterpene isolated from the sponge *Acanthella klethra*. This compound showed antituberculosis activity against *M. tuberculosis* with an MIC of 2.0 μg/mL along with promisingly low cytoxicity (IC_{50} > 20 μg/mL against KB cells).



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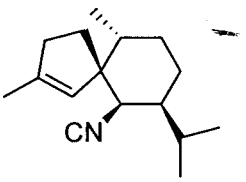
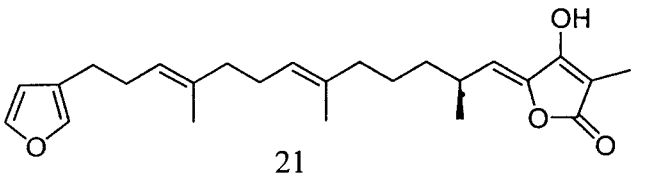


20

1.3 The sesterterpenoids

The sesterterpenoids arise from geranylfarnesyl diphosphate (GFPP), which is formed by addition of a further isopentenyl diphosphate (IPP) molecule to geranylgeranyl diphosphate (GGPP). With an extensive examples of compounds in this group that are now known, most are nevertheless found principally in fungi and marine organisms, and span relatively few structural types (Dewick, 1997). In fact, the sesterterpenes can be classified into only six main types, including linear, mono-, bi-, tri, tetra-carbocyclic and fungal sesterterpenoids. Some representative examples of each class are shown below.

Marine sponges have been the major sources of a large number of linear sesterterpenoids. Many of these compounds contain a furan ring and a tetronic acid moiety while most of the remainings are the previous group's degradation products. For example, variabilin **(21)** isolated from the sponge *Sarcotragus* sp. (Barrow *et al.*, 1988) and konakhin **(22)** isolated from a Senegalese sponge represents a degradation product of the tetronic acid (N'Diaye *et al.*, 1991).



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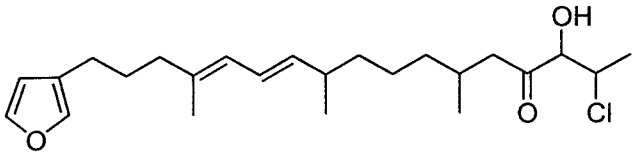
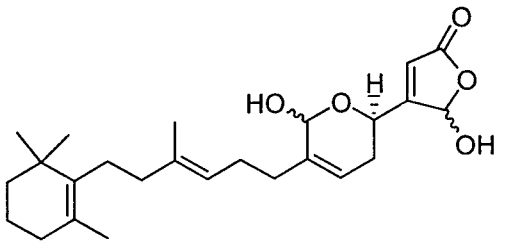


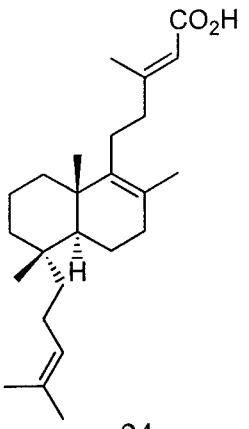
22

Mono-carbocyclic sesterterpenoids are examplified by manoalide **(23)** and its derivatives. Manoalide significantly reduces chemically induced inflammation and was originally found in the sponge *Luffariella variabilis* (de Silva and Scheuer, 1980; Jacobs *et al.*, 1985).



23

Dysideapalaunic acid **(24)** and aplysolide A **(25)** are, respectively, examples of bi- and tri-carbocylic sesterterpenoids. Dysideapalaunic acid was isolated from the sponge *Dysidea* sp., and showed the inhibition toward aldose reductase (Hagiwara and Uda, 1991). Aplysolide A is hydroxy-butenolides obtained from a sponge *Aplysinopsis* sp. (Crews, Jimenez and Neil-Johnson, 1991).



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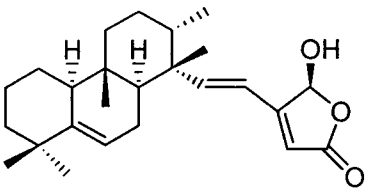
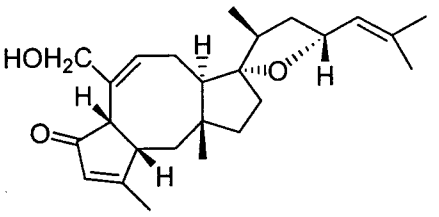


25

The scalaranes, which belongs to the tetracarbocyclic type, are the most common sesterterpenoids and most extensively studied compounds. The details regarding their chemistry and bioactivities will be discussed in the next section of this chapter.

The last group, the fungal sesterterpenoids, are mainly produced by plant fungal pathogens of the genus *Drechslera*. Ophiobolin A **(26)**, for example, is a phytotoxic metabolite isolated from *Drechslera sorghicola*, which is a pathogen on sorghum and Johnson grass (Sugawara *et al.*, 1988).



26

1.3.1 Scalarane-type sesterterpenoids

Scalarane-type sesterterpenes are found widely distributed in several marine sponge species, especially those from the family *Dictyoceratida* (Hanson, 1992). Certain members of this group can also be found in nudibranches, which assoicate with the sponges containing these compounds. The scalarane skeleton is shown in Figure 2.

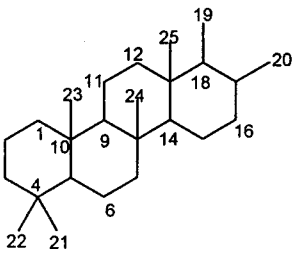


Figure 2 Scalaranes skeleton

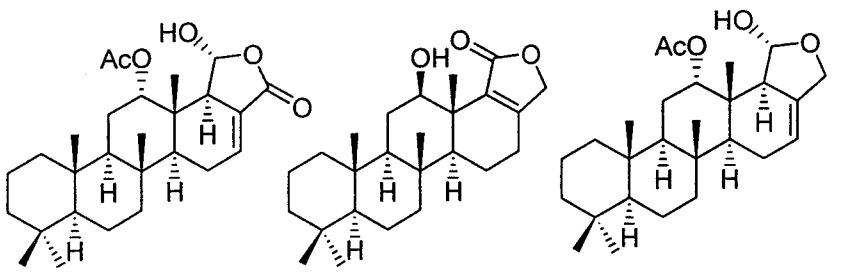
To date, there are up to more than 100 naturally occuring scalarane-type sesterterpenes reported, which can be classified into two categories, furanoscalaranes and non-furanoscalaranes.

1.3.1.1 Furanoscalaranes

Constructing the major category, most scalarane-type sesterterpenes reported to date are belonging to the furanoscalaranes. The main skeleton of the members in this class possesses a tetracarbocyclic ring fused with an extended furan moiety onto C17-C18 of ring D. The oxidation state, as well as the joining positions, of the furan residue are varied, from a simple hydrofuran, to aromatic

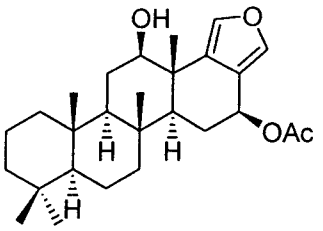
and oxygenated furans. Some selected prototypes of the furanoscalaranes are examplified below.

The oxygenating degree in the lactone moiety varies from hydroxy lactone, as seen in scalarin **(27)** from the sponge *Cacospongia scalaris* (Fattorusso *et al.*, 1972), to simple lactone, as seen in scalarolide **(28)** from the sponge *Spongia idia* (Walker, Thompson and Faulkner, 1980) and lactol as seen in heteronemin **(18)** from the sponge *Heteronema erecta* (Kazlauskas *et al.*, 1976) and deoxoscalarin **(29)** from the sponge *Spongia officinalis* (Cimino *et al.*, 1977). Normally, the oxygenating position is found at either C-19 or C-20.



27 28 29

The non-oxygenated furano type, although found less frequently, was also reported. The prototype of such group include scalarafuran **(30)**, from the sponge *Spongia idia* (Walker, Thompson and Faulkner, 1980), of which the extended furan-subunit is the fully aromatized. Also rare were rearranged furanoscalaranes, in which the furan moiety is otherwise attached on its *b*-face, suggesting an oxidative cleavage-recyclization biosynthetic scheme. The example of such furanoscalarane is furoscalarol **(31)** from the sponge *Cacospongia mollior* (Cimino *et al.*, 1978).



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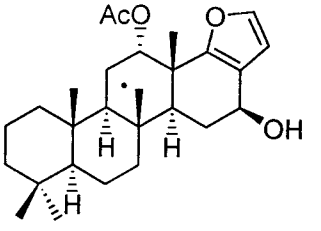
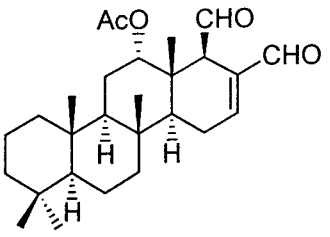


31

1.3.1.2 Non-furanoscalaranes

The members of this group belong to the tetracarbocyclic scalaranes with no furan residue on ring D. Most often, the functional group variation is found substituted at C-12, C-16, C-19 and C-20. The prototype of this group is scalaradial **(32),** which was first isolated from the sponge *Cocospongia mollior* (Cimino *et al.*, 1974), and sednolide **(33)** from the nudibrance *Chromodoris sedna* (Hochlowski and Faulkner, 1983).

Additionally, there are some other members that incorporate structural subunit from other biosynthetic pathway. These include disidein **(34),** pentacyclic scalaranes combined with a hydroxyhydroquinone ring, isolated from the sponge *Disidea pellscens* (Cimino *et al.*, 1975). The hydroquinone residue of **34** is clearly demonstrating the involvement of triketide intermediate during the biosynthetic pathway.



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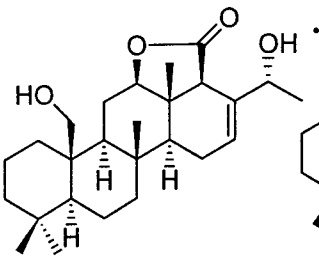

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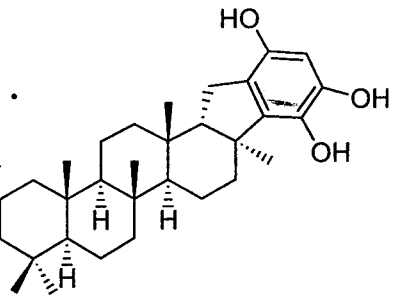


34

The activities and biological sources of all members in scalarane-type sesterterpenes reported to date are summarized in Table 2.

Table 2 Biological sources and activities of scalarane-type sesterterpenoids

Compounds	Sources	Activities	References
1. Furanoscalaranes			
1.1 furanone-type			
Scalarin	*Cacospongia scalaris* (sponge)	N/A	Fattorusso *et al.*, 1972
12-Epi-scalarin	*Spongia nitens* (sponge)	N/A	Cimino *et al.*, 1977
Scalarolide	*Spongia idia* (sponge)	N/A	Walker *et al.*, 1980
23-Hydroxy-20-methylscalarolide	*Chromodoris sedna* (nudibranch)	N/A	Hochlowski and Faulkner, 1983
Phyllofolactone A	*Phyllospongia foliascens* (sponge)	N/A	Zeng *et al.*, 1991

Table 2 (cont.)

Compounds	Sources	Activities	References
Phyllofolactone B	*Phyllospongia foliascens* (sponge)	N/A	Zeng *et al.*, 1991
Phyllofolactone B acetate	*Carteriospongia foliascens* (sponge)	N/A	Barron *et al.*, 1991
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
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.*, 1993
Phyllactone G	*Phyllospongia foliascens* (sponge)	N/A	Fu *et al.*, 1993
12-*O*-Deacetyl scalarin	*Hyrtios* sp. (sponge)	Nerve growth factor synthesis-stimulating (concentration 30-100 μg/mL)	Doi *et al.*, 1993

Table 2 (cont.)

Compounds	Sources	Activities	References
16-*O*-Deacetyl-16-episcalarol butenolide	*Hyrtios erecta* (sponge)	Cytotoxic (IC_{50} 0.4 μg/mL against P-388)	Ryu *et al.*, 1996
12-*O*-Deacetyl-16-*O*-deacetyl-16-epi scalarolbutenolide	*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} 0.4 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
12-Deacetyl-12-epi-19-deoxyscalarin	*Hyrtios erecta* (sponge)	Cytotoxic (ED_{50} 2.9 μg/mL against P-388)	Pettit *et al.*, 1998
Sesterstatin 1	*Hyrtios erecta* (sponge)	Cytotoxic (ED_{50} 0.46 μg/mL against P-388)	Pettit *et al.*, 1998
Sesterstatin 2	*Hyrtios erecta* (sponge)	Cytotoxic (ED_{50} 4.2 μg/mL against P-388)	Pettit *et al.*, 1998
Sesterstatin 3	*Hyrtios erecta* (sponge)	Cytotoxic (ED_{50} 4.3 μg/mL against P-388)	Pettit *et al.*, 1998

Table 2 (cont.)

Compounds	Sources	Activities	References
12-*O*-Acetyl-16-*O*-deacety-12,16-epi scalarolbutenolide	*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
Hyrtiolide	*Hyrtios erecta* (sponge)	N/A	Miyaoka *et al.*, 2000
16-Hydroxy scalarolide	*Hyrtios erecta* (sponge)	N/A	Miyaoka *et al.*, 2000
Phyllofolactones H	*Strepsichordaia aliena* (sponge)	N/A	Jimenez *et al.*, 2000
Phyllofolactones I	*Strepsichordaia aliena* (sponge)	N/A	Jimenez *et al.*, 2000
Phyllofolactones J	*Strepsichordaia aliena* (sponge)	N/A	Jimenez *et al.*, 2000
Phyllofolactones K	*Strepsichordaia aliena* (sponge)	N/A	Jimenez *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
1.2 furanol-type			
Deoxoscalarin	*Spongia officinalis* (sponge)	N/A	Cimino *et al.*, 1977

Table 2 (cont.)

Compounds	Sources	Activities	References
12-Epi-deoxoscalarin	*Spongia nitens* (sponge)	N/A	Cimino *et al.*, 1977
Heteronemin	*Heteronema erecta* (sponge)	Cytotoxic (IC_{50} 1.2 μg/mL against KB); Antituberculosis (MIC 6.25 μg/mL against *M. tuberculosis* ($H_{37}Rv$)	Kazlauskas *et al.*, 1976; Doi *et al.*, 1993; El *sayed et al.*, 2000
Scalardysin A	*Dysidea herbacea* (sponge)	N/A	Kashman and Zviely, 1979
Scalardysin B	*Dysidea herbacea* (sponge)	N/A	Kashman and Zviely, 1979
12-Deacetyl-20-methyl-12-epideoxoscalarin	*Chromodoris sedna* (nudibranch)	N/A	Hochlowski and Faulkner, 1983
23-Hydroxy-20-methyldeoxoscalarin	*Chromodoris sedna* (nudibranch)	N/A	Hochlowski and Faulkner, 1983
12-α-Acetoxy-19,20-epoxy-20-hydroxy-20,22-dimethyl scalarane	*Carteriospongia foliascens* (sponge)	Ichthyotoxic (LD_{50} 40 mg/L against *Lebistes reticulatus*)	Braekman *et al.*, 1985
Heteronemin acetate	*Hyrtios erecta* (sponge)	N/A	Crews and Bescansa, 1986
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	Karuso *et al.*, 1989
12-Epi-heteronemin	*Hyrtios erecta* (sponge)	N/A	Bourguet-Kondracki *et al.*, 1994
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
21-Acetoxydeoxo scalarin	*Chromodoris inornata* (nudibranch)	Cytotoxic (IC_{50} 0.35 μg/mL against L1210)	Miyamoto *et al.*, 1999
21-Hydroxydeoxo scalarin	*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-Deacetyl-12-epi-deoxoscalarin	*Glossodoris atromarginata* (nudibranch)	N/A	Fontana *et al.*, 1999

Table 2 (cont.)

Compounds	Sources	Activities	References
12-Deacetyl-23-acetoxy-20-methyl 12-epi-deoxoscalarin	*Glossodoris sedna* (nudibranch)	N/A	Fontana *et al.*, 2000
1.3 non-oxygenated furan-type			
Furoscalarol	*Cacospongia mollior* (sponge)	N/A	Cimino *et al.*, 1978
Scalarafuran	*Spongia idia* (sponge)	Cytotoxic (IC_{50} 7.2 μg/mL against KB)	Walker *et al.*, 1980; Doi *et al.*, 1993
16-Deacetyl-12-epi-scalafuran acetate	*Spongia officinalis* (sponge)	N/A	De Giulio *et al.*, 1989
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
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-*O*-Desacetyl furoscalar-16-one	*Cacospongia sp* (sponge).	N/A	Cambie *et al.*, 1998
Salmahyrtisol B	*Hyrtios erecta* (sponge)	N/A	Youssef *et al.*, 2002

Table 2 (cont.)

Compounds	Sources	Activities	References
2. Non-furanoscalaranes			
Scalaradial	*Cocospongia mollior* (sponge)	Brine shrimp lethality (LD_{50} 0.18 μg/mL); Fish antifeedant (MIC 60 μg/cm^2 against *Carassius carassius*); Inhibited PLA_2 (IC_{50} 0.6 μM)	Cimino *et al.*, 1974; De Rosa *et al.*, 1994; Fontana *et al.*, 2000
Disidein	*Disidea pellscens* (sponge)	N/A	Cimino *et al.*, 1975
12-Epi-scalaradial	*Spongia nitens* (sponge)	N/A	Cimino *et al.*, 1979
12,18-Diepi-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
12-Deacetyl-12,18-diepi-scalaradial	*Spongia idia* (sponge)	N/A	Walker *et al.*, 1980

Table 2 (cont.)

Compounds	Sources	Activities	References
12-Deacetyl scalaradial	*Cacospongia scalaris* (sponge)	Cytotoxic (ED_{50} 0.58 μg/mL against L-1210)	Yasuda and Tada, 1981
Foliaspongin	*Phyllospongia foliascens* (sponge)	Antiinflammatory (18.1% inhibition at concentration 10 μg/disk utilizing chrio-allantoic membrane of chick embryo	Kikuchi *et al.*, 1983
Sednolide	*Chromodoris sedna* (nudibranch)	N/A	Hochlowski and Faulkner, 1983
Sednolide-23-acetate	*Chromodoris sedna* (nudibranch)	N/A	Hochlowski and Faulkner, 1983
Hyrtial	*Hyrtios erecta* (sponge)	Antiinflamatory (43% decrease in ear weight of PMA induced inflammation 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

Table 2 (cont.)

Compounds	Sources	Activities	References
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-oxoscalaradial	*Chromodoris youngbleuthi* (nudibranch)	N/A	Terem and Scheuer, 1986
Triacetyl disidein	*Disidea pallescens* (sponge)	N/A	Cimino *et al.*, 1987
6′-Cl-disidein	*Disidea pallescens* (sponge)	N/A	Cimino *et al.*, 1987
6′-Br-disidein	*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)	Kitagawa *et al.*, 1989
Dehydrofoliaspongin	*Phyllospongia foliascens* (sponge)	N/A	Kitagawa *et al.*, 1989
Phyllofenone A	*Phyllospongia foliascens* (sponge)	N/A	Zeng *et al.*, 1991

Table 2 (cont.)

Compounds	Sources	Activities	References
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 $\mu g/cm^2$ 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
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} 2 μg/mL against P-388, Schabel, A-549, HT-29 and MEL-28)	Rueda *et al.*, 1997

Table 2 (cont.)

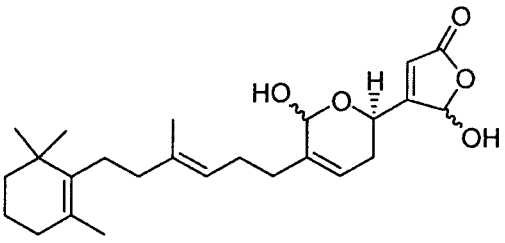
Compounds	Sources	Activities	References
Norscalaral C	*Cacospongia scalaris* (sponge)	Cytotoxic (ED_{50} 1.2-2.5 μg/mL against P-388, Schabel, A-549, HT-29 and MEL-28)	Rueda *et al.*, 1997
25,26-Bishomo-scalarane	*Cacospongia scalaris* (sponge)	N/A	De Rosa *et al.*, 1998
12-Deacetyl-Δ^{17}-hyrtial	*Hyrtios erectus* (sponge)	Antiproliferative (IC_{50} 2.82 μg/mL against KB)	Miyaoka *et al.*, 2000
Honu'enone	*Strepsichordaia aliena* (sponge)	N/A	Jimenez *et al.*, 2000
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 affinis*); Inhibited PLA_2 (IC_{50} 18 μM)	Fontana *et al.*, 2000

Note: N/A = not available

1.3.2 Manoalide-type sesterterpenoids

The members in this class are monocarbocyclic sesterterpenoids normally containing butenolide end. Most of them have been reported from the sponge *Luffariella variabilis.* Their bioactivities are mostly antiinflammatory. Substituted position on butenolide moiety can be used to classify this group into two major types, including β-substituted- and γ-substituted-butenolide-type sesterterpenes.

Manoalide **(23)** is the prototype of alkylated trimethyl- cyclohexenyl with β-substituted-α,β-unsaturated-γ-hydroxybutenolide moiety. The compound was first isolated from a sponge *Luffariella variabilis* (de Silva and Scheuer, 1980) and was reported to reduce chemically induced inflammation. Seco-manoalide **(35)** possesses the same structural type but lack of cyclized α,β-unsaturated δ-lactol moiety and was isolated from the same sponge species (de Silva and Scheuer, 1981).



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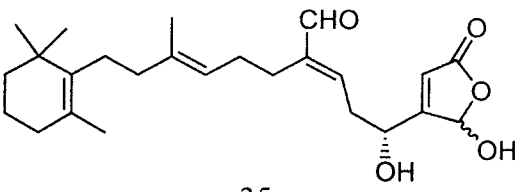

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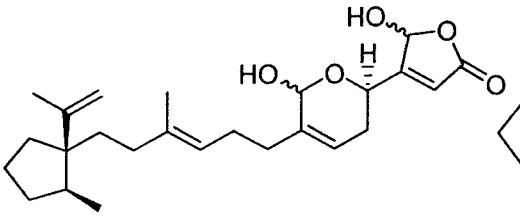
Luffariellins A **(36)** and B **(37)** are geometric isomers of **23** and **25**, respectively, and were also isolated from the same sponge species (Kernan and Faulkner, 1987). These compounds possess alkylated cyclopentanyl with β-substituted-α,β-unsaturated-γ-hydroxybutenolide moiety.



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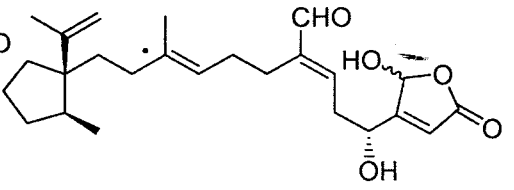

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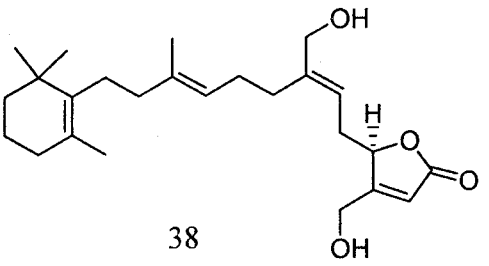
The γ-substituted-butenolide-type sesterterpenes are examplified by *E*- and *Z*-neomanoalide (**38** and **39**, respectively). These compounds also possess the alkylated trimethylcyclohexenyl group with β,γ-disubstituted-α,β-unsaturated-butenolide moiety.



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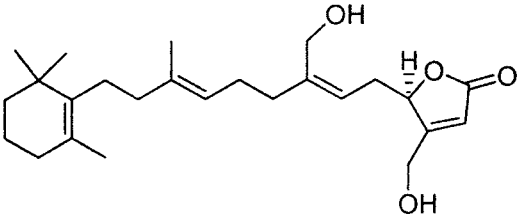

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The activities and biological sources of all members are summarized in Table 3.

Table 3 Biological sources and activities of manoalide-type sesterterpenoids

Compounds	Sources	Activities	References
1. β-substituted-butenolide-type			
Manoalide	*Luffariella varabilis*	Antiinflammatory (inactivated directly PLA_2); Analgesic; Prevent paralytic action of β-bungarotoxin on the rat phrenic nerve-hemidiaphragm preparation; Cytotoxic (IC_{50} 0.022 and 0.26 μg/mL against L1210 and KB, respectively)	de Silva and Scheuer, 1980; de Freitas *et al.*, 1984; Jacobs *et al.*, 1985; Kobayashi *et al.*, 1994
Seco-manoalide	*Luffariella varabilis*	Antiinflammatory, inhibit aldose reductase (82% inhibition with MIC 2×10^{-6} M)	de Silva and Scheuer, 1981; Katsumura *et al.*, 1987
Luffariellin A	*Luffariella varabilis*	Antiinflammatory (IC_{50} 5.6×10^{-8} M against bee venom PLA_2)	Kernan and Faulkner, 1987
Luffariellin B	*Luffariella varabilis*	Antiinflammatory (IC_{50} 6.2×10^{-8} M against bee venom PLA_2)	Kernan and Faulkner, 1987

Table 3 (cont.)

Compounds	Sources	Activities	References
Manoalide 25-acetate	*Thorectandra excavatus*	N/A	Cambie and Craw,1988
Thorectolide 25-acetate	*Thorectandra excavatus*	N/A	Cambie and Craw,1988
Luffariellolide	*Fascaplysinopsis* sp.	N/A	Roll *et al.*, 1988
Dehydro luffariellolide diacid	*Fascaplysinopsis reticulata*	N/A	Jimenez *et al.*, 1991
Luffariolide A	*Luffariella* sp.	Cytotoxic (IC_{50} 1.1 μg/mL against L1210)	Tsuda *et al.*, 1992
Luffariolide B	*Luffariella* sp.	Cytotoxic (IC_{50} 1.3 μg/mL against L1210)	Tsuda *et al.*, 1992
Luffariolide D	*Luffariella* sp.	Cytotoxic (IC_{50} 4.2 μg/mL against L1210)	Tsuda *et al.*, 1992
Luffariolide E	*Luffariella* sp.	Cytotoxic (IC_{50} 1.2 μg/mL against L1210)	Tsuda *et al.*, 1992
2. γ-substituted-butenolide-type			
E-Neomanoalide	*Luffariella varabilis*	Cytotoxic (IC_{50} 9.8 μg/mL against L1210)	de Silva and Scheuer, 1981; Tsuda *et al.*, 1992

Table 3 (cont.)

Compounds	Sources	Activities	References
Z-Neomanoalide	*Luffariella varabilis*	Cytotoxic (IC_{50} 5.6 μg/mL against L1210)	de Silva and Scheuer, 1981; Tsuda *et al.*, 1992
Luffariolide C	*Luffariella* sp.	Cytotoxic (IC_{50} 7.8 μg/mL against L1210)	Tsuda *et al.*, 1992
Z-2,3-Dihydro neomanoalide	*Luffariella* sp.	Antibacterial (MIC 1 and 5 μg/mL against *Escherichia coli* and *Bacillus subtilis*, respectively	Konig *et al.*, 1992
Z-24-Acetoxy-2,3-dihydro neomanoalide	*Luffariella* sp.	Antibacterial (MIC 3 and 11 μg/mL against *B. subtilis and Micrococcus luteus*, respectively	Konig *et al.*, 1992
Z-24-Acetoxy neomanoalide	*Luffariella* sp.	Antibacterial (MIC 8 and 2 μg/mL against *B. subtilis* and *M. luteus*, respectively	Konig *et al.*, 1992
E-Neomanoalide - 24-al	*Luffariella* sp.	Antibacterial (MIC 4 μg/mL against *B. subtilis* and *M. luteus*	Konig *et al.*, 1992

Table 3 (cont.)

Compounds	Sources	Activities	References
Luffarin-P	*Luffariella geometrica*	N/A	Butler and Capon, 1992

Note: N/A = not available

1.4 The Genus *Brachiaster*

The identification of sponge species is not easy even for experts and requires special technique. Sponges are taxonomically classified by means of skeleton structures (spicule and spongin), external characteristics (shape, size, color, texture, mucous production, smell) and biochemical, reproductive and ecological characteristics. They are taxonomically classified into four classes; Demospongiae, Hexactinellida, Calcarea and Sclerospongiae (Bergquist, 1978; Hooper, 2000). Approximately 95% of sponges are in the class Demonspongiae, which the genus *Brachiaster* belongs to (Hopper, 2000).

The taxa of this Genus is as followed; Phylum Porifera, Class Demospongiae, Order Choristida, Family Pachastrellidae and Genus *Brachiaster*.

Hooper (2000) described the characteristic of the Family Pachastrellidae as following;

> ...Encrusting, massive and plate-shaped growth forms, with ostia and oscules on opposite sides; megascleres calthrops, short-shafted triaenes, and oxeas; microscleres streptasters of various types (metasters, spirasters and amphiasters), but never euasters; desmas common in some genera ('lithistid' or 'sublithistid' grades of construction). Seventeen genera are included for this family...

To our knowledge, there are no reports regarding chemical constituents from the sponges of the genus *Brachiaster*.

CHAPTER 2

EXPERIMENTAL

2.1 General

Unless stated otherwise, solvents for general purposes were commercial grade and were re-distilled prior to use. All preparative HPLC solvents were analytical grade and were filtered through membrane filter 0.45 μm and degassed by ultrasonic sonicator prior to use. Analytical TLC was performed on Merck® pre-coated siliga gel 60 F_{254} plates (layer thickness 0.20 mm). Visualization was accomplished by observation under UV light (254 nm) and by staining with phosphomolybdic acid (10% solution in ethanol) followed by heating. The size-exclusion chromatography was conducted on a column of Sephadex® LH-20, which was allowed to saturate with eluting solvents as indicated for an overnight prior to use. Flash column chromatography was carried out using Merck® siliga gel 60 (particle size 0.04-0.06 mm, 230-400 mesh ASTM), according to the procedure described by Still, Kahn and Mitra (1978).

HPLC was performed on Waters® multisolvent delivery system (model 600E) connected to Waters® tunable absorbance detector (model 486). This was equiped with a Rheodyne® injector port (model 7125). Reverse phase HPLC was performed using Thermo Hypersil® BDS C_{18} (5 μ, 250×4.6 mm) or Hamilton®

PRP-1 semi-preparative (10 μ, 305×7.0 mm) C_{18} polymer-based columns. The normal phase HPLC column used was Econosil® semi-preparative (10 μ, 250×7.0 mm) column.

NMR spectra were recorded on a FTNMR, Varian Unity® Inova 500 spectrometer (500 MHz for proton and 125 MHz for carbon-13). The chemical shifts were reported on the δ-scale relative to the solvent signals. The operating NMR solvents used were benzene-d_6 (7.15 ppm of residual C_6HD_5 for ^1H NMR and 128.0 ppm for ^{13}C NMR) and chloroform-*d* (7.24 ppm of residual $CHCl_3$ for ^1H NMR and 77.0 ppm for ^{13}C NMR). Signal multiplicities were indicated by s, d, t, br, and m; denoting singlet, doublet, triplet, broad, and multiplet, respectively. IR spectra were recorded on a Jasco® IR-810 infrared spectrometer. UV spectra were obtained from a Spectronic® Genesys 5 spectrophotometers. Mass spectra were obtained from a Micromass® LCT mass spectrometer or HP 5890 GC series 2 plus-HP 5972 mass selective detector. Optical rotation and CD spectra were recorded in methanol on a Jasco® J-810 spectropolarimeter, using sodium D-line wavelength at 589 nm.

2.2 Sponge material

The sponge (Figure 3) was collected at the depth of 18-20 meters from Koh-Tao, Surat Thani, Thailand in April 2001, and in April 2002. It was identified as *Brachiaster* sp. (Order Astrophorida, Family Pachastrellidae) by Mr.Somchai Busaravich of Phuket Marine Biological Center. The sponge is lumpy- and

khaki internally. The outer texture is prickly, with tiny unpenetrating spines covering over the surface. When touched, the specimen appears very tough, incompressible, and resistible to be cut. The sponge voucher specimen (AP 01-008-03) was preserved in 70% ethanolic solution and was deposited at Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University. The remaining specimens were preserved at –20 °C until the time of extraction.

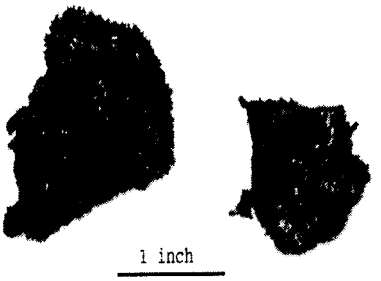


Figure 3 The Thai sponge, *Brachiaster* sp.

2.3 Bioactivity determination

2.3.1 Antituberculosis activity

The antituberculosis activity was assessed against *Mycobacterium tuberculosis* $H_{37}Ra$ using the Microplate Alamar Blue Assay (MABA) (Collins and Franzblau, 1997). The activity determination was kindly supported by Dr.Prasat Kittakoop of the National Center for Genetic Engineering and Biotechnology.

Initial sample dilutions were prepared in either DMSO or distilled deionized water, and subsequent two-fold dilutions, starting from 200 μg/mL, were performed in 0.1 mL of 7H9GC (no Tween 80) in the microplate. BACTEC 12B-passaged inocula were initially diluted 1:2 in 7H9GC, and 0.1 mL 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 1:20 in BACTEC 12 B medium followed by a 1:50 dilution in 7H9GC. Addition of 1/10 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 at 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 h for a color change from blue to pink. If the B wells became pink within 24 h, 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 reagents were added to all remaining wells. Plates were then incubated at 37°C and resulted colors were recorded at 24 h post-reagent addition. For the positive control, isoniazid and kanamycin sulfate were used as standard drugs. The MICs of both agents in the test system were 0.040-0.090 and 2.0-5.0 μg/mL, respectively.

Visual MICs were defined as the lowest concentration of samples that prevented a color change.

2.3.2 Cytotoxic activity

The determination was kindly supported by Assist. Prof. Supreeya Yuenyongsawad of Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University. The cell lines utilized as the target cells in this test were MCF-7 (breast adenocarcinoma), HeLa (human cervical cancer), HT-29 (colon cancer) and KB (human oral cancer). 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 of each cell line 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 of 72 h). 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 solubilized 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 obtained by plotting the survival percentage against the concentrations of tested samples.

2.4 Isolation and purification

The freeze-dried sponge (158.60 g) from the first collection was crushed and macerated exhaustively (5×1.5 L) in methanol to yield a crude extract, which was then subjected to a solvent partitioning scheme using the procedure modified from Kupchan and Tsou *et al.* (1973). Hexane-, CH_2Cl_2- and *n*-BuOH-soluble materials were obtained (1.11 g, 0.70%; 1.64 g, 1.03%; 1.32 g, 0.83%, respectively). The hexane and CH_2Cl_2 fractions, which exhibited antituberculosis activity against *Mycobacterium tuberculosis* $H_{37}Ra$ with MIC of 6.25 μg/mL, were chosen for the isolation of the bioactive compounds (Scheme 1).

The CH_2Cl_2 fraction was fractionated by the chromatographic technique using a column of Sephadex LH-20 with methanol as eluent. The eluates were collected approximately 20 mL per fraction. Fractional pool, monitored by TLC technique (10% MeOH in CH_2Cl_2 as developing system) and comfirmed by antituberculosis assay, led to two major active fractions (MICs 6.25 and 3.125 μg/mL). These active fractions were further purified separately over a SiO_2 column (3% EtOAc in CH_2Cl_2), then repeatedly re-crystallized to yield compound **18** as white needles (99 mg, 6.0% of CH_2Cl_2 fraction), which was later identified as heteronemin.

The hexane fraction, which was also active, was chromatographed over a SiO_2 column (5% EtOAc in CH_2Cl_2). Fraction combination, achieved as stated above yield two active fractions (MIC 0.39 μg/mL). Each was separately further purified using reverese phase HPLC (5 μ, 250×4.6 mm) with isocratic 87% aqueous CH_3CN (flow rate 1 mL/min, UV detector, 220 nm). Two white non-crystallized compounds, **41** (10 mg, 0.9% of hexane fraction) and **42** (2 mg, 0.2% of hexane fraction), eluted at retention times 18.0 and 14.8 min, respectively, were collected. They were identified as heteronemin acetate and 12-epi-19-deoxyscalarin, respectively.

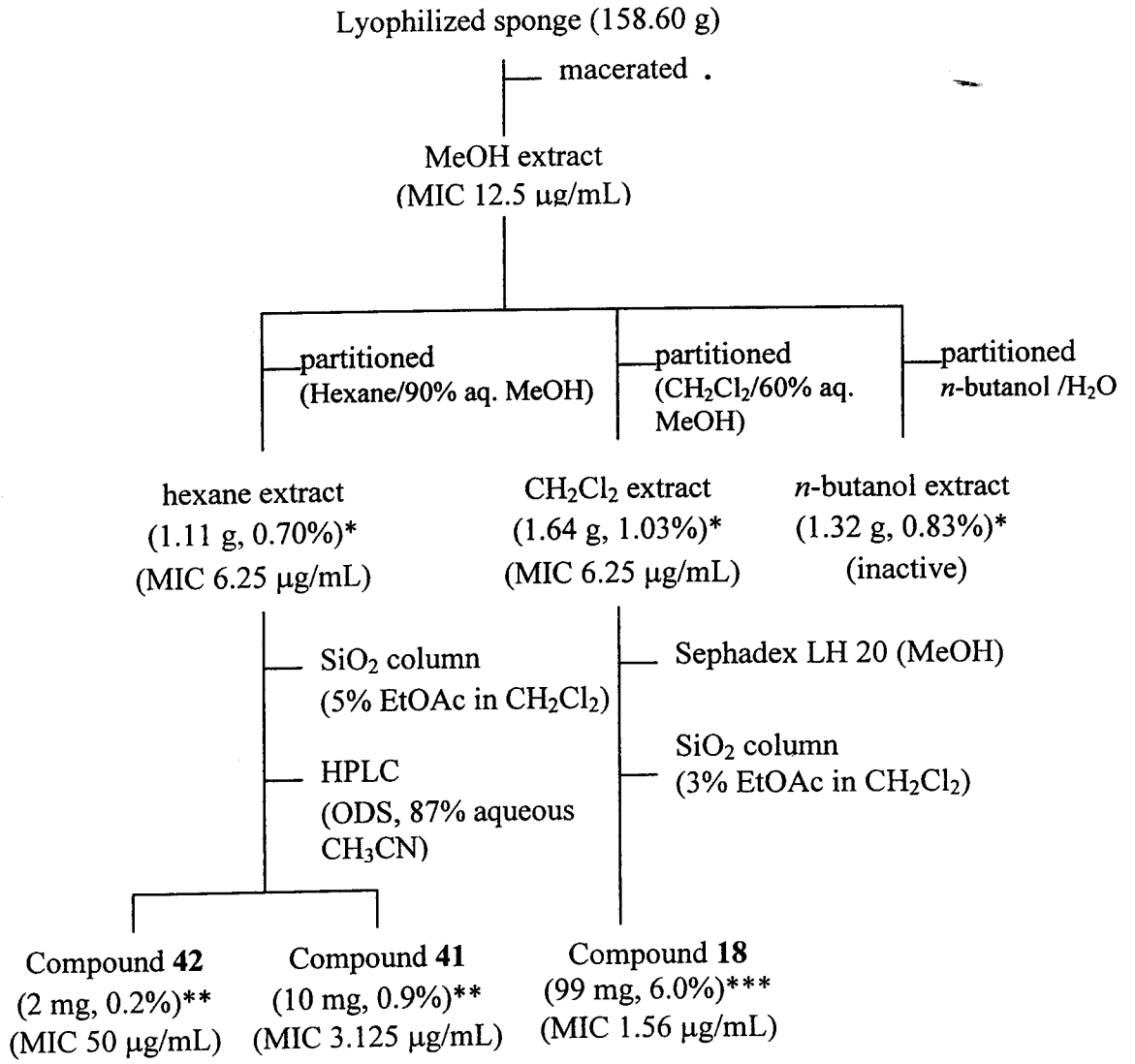
The freeze-dried sponge (212.33 g) from the second expedition was crushed and consecutively extracted with hexane (3×2 L), CH_2Cl_2 (3×2 L) and MeOH (3×2 L) (13.49 g, 6.35%; 1.36 g, 0.64%; 26.90 g, 12.67%, respectively). The large hexane fraction, which also exhibited antituberculosis activity (MIC of 3.125 μg/mL), was chosen for the further isolation of the bioactive compounds (Scheme 2).

An aliquot of the hexane fraction (4.30 g) was isolated by the flash chromatographic technique over a column of SiO_2 (20:5:75 of EtOAc:acetone: hexane). Fractions with similar chromatographic pattern were combined to yield four main fractions. The first fraction was re-crystallized with CH_2Cl_2-methanol mixture (1:3) to afford compound **18** (390 mg, 9.1% of hexane fraction), which was identical to that obtained from the previous expedition, was identified as heteronemin.

The second fraction was further fractionated using semi-preparative normal phase HPLC (10 μ, 250×7.0 mm) with isocratic 5% isopropanol in hexane as mobile phase (flow rate 2 mL/min; UV detector, 220 nm) to afford compound **43** (2 mg, 0.05% of hexane fraction, T_R 17.5 min) as white needles. It was identified as 12-deacetyl-12-epi-19-deoxyscalarin. The residue from this fractionation step was combined and further purified using reverese phase HPLC (5 μ, 250×4.6 mm) with isocratic 75% aqueous CH_3CN as mobile phase (flow rate 1 mL/min; UV detector, 220 nm). Two viscous colorless liquids, compounds **44** and **45** (4 and 7 mg; 0.1 and 0.2% of hexane extract, respectively) were obtained at 24.5 and 28.3 min. They were identified as (*E*) and (*Z*)-neomanoalide diacetates, respectively.

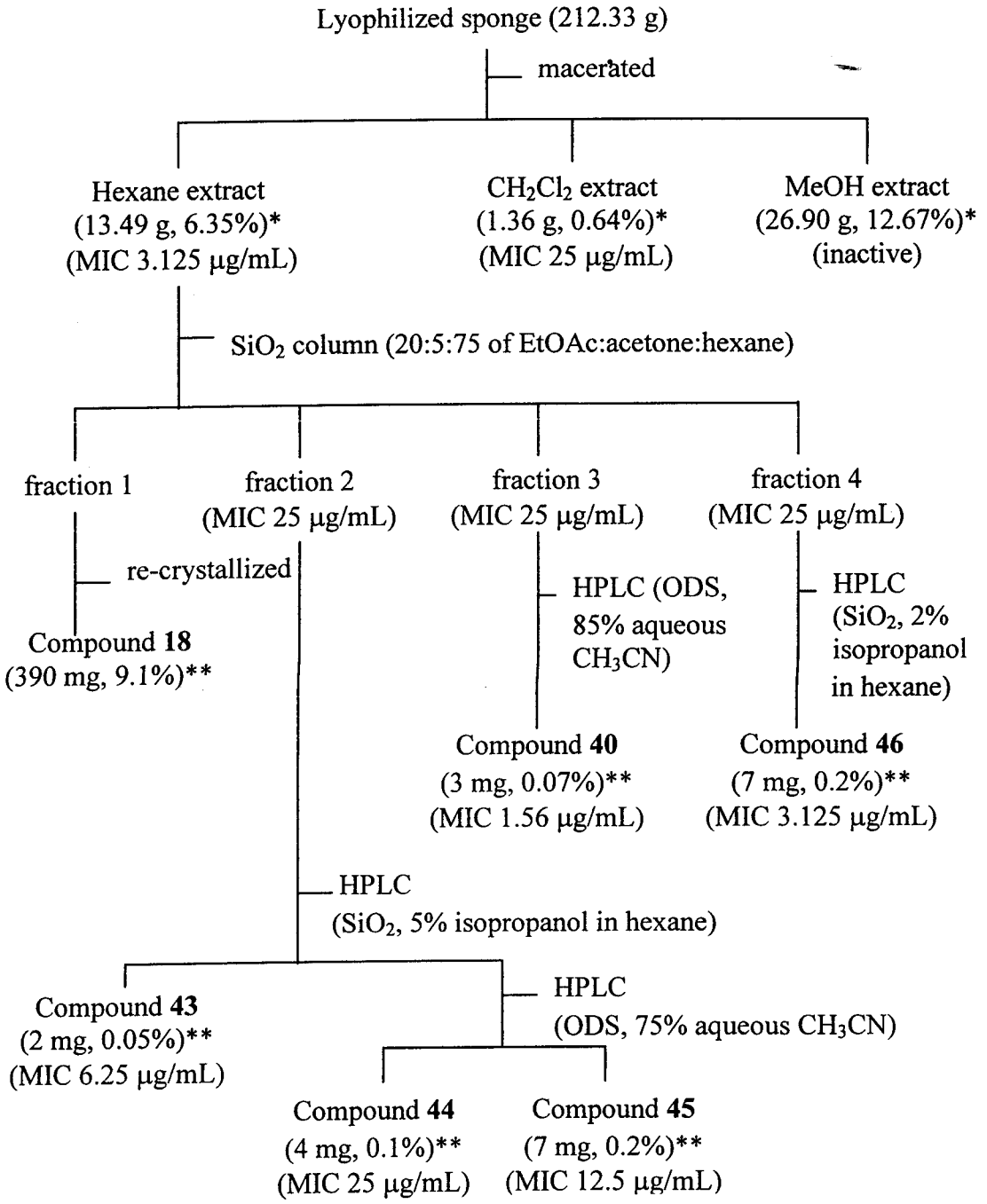
The third fraction was further purified using reverese phase HPLC (5 μ, 250×4.6 mm) with isocratic 85% aqueous CH_3CN as mobile phase (flow rate 1 mL/min; UV detector, 220 nm). A white non-crystallized compound, compound **40** (3 mg, 0.07% of hexane fraction), was obtained from the eluate at 27.1 min. It was identified as 12-deacetoxy-scalarin acetate. Along with **40**, the presence of **41** and **42** was also observed via TLC detection. The two compounds, however, were not further isolated in this step.

The last fraction was further isolated using semi-preparative normal phase HPLC (10 μ, 250×7.0 mm) with isocratic 2% isopropanol in hexane as mobile phase (flow rate 2 mL/min; UV detector, 220 nm) to give compound **46** (7 mg, 0.2% of hexane fraction, T_R 21.5 min) as white needles (re-crystallized from 1:5 of isopropanol:hexane mixture). It was identified as manoalide-25-acetate.



* % of dried weight of sponge
* isolated yield, % of hexane extract
**isolated yield, % of CH_2Cl_2 extract

Scheme 1 Extraction and isolation scheme of the Thai sponge, *Brachiaster* sp. (April 2001 expedition)



* % of dried weight of sponge
** isolated yield, % of an aliquot of hexane extract

Scheme 2 Extraction and isolation scheme of the Thai sponge, *Brachiaster* sp. (April 2002 expedition)

2.5 Physical properties of isolated compounds

12-Deacetoxy-scalarin acetate (40): white amorphous solid; $[\alpha]_D^{25}$ –24.3° (c=0.014, MeOH); IR (thin film) ν_{max} 1770, 1730 cm^{-1}; UV (MeOH) λ_{max} (log ε) 227 (3.78) nm; CD (c=1.58×10^{-4} M, MeOH) θ (nm) 0 (272), +2177 (251), 0 (239); ^1H and ^{13}C NMR (500 MHz for ^1H, C_6D_6) see Table 4; ESIMS *m/z* (relative intensity) 429 ($[M+H]^+$, 29), 407 (29), 369 (100), 358 (29), 336 (29); HREIMS *m/z* 428.2910 [$C_{27}H_{40}O_4$ requires 428.2927].

Heteronemin (18): white needles (CH_2Cl_2:methanol = 1:3); $[\alpha]_D^{20}$ –71.4° (c=0.055, $CHCl_3$); IR (thin film) ν_{max} 3500, 1740, 1235 cm^{-1}; UV (MeOH) λ_{max} (log ε) 229 (2.34) nm; ^1H and ^{13}C NMR (500 MHz for ^1H, $CDCl_3$) see Table 5; ESIMS *m/z* (relative intensity) 511 ($[M+Na]^+$, 23), 429 (13), 369 (100), 351 (54), 191 (13), 148 (8).

Heteronemin acetate (41): white amorphous solid; $[\alpha]_D^{20}$ –48.6° (c=0.075, $CHCl_3$); IR (thin film) ν_{max} 1740, 1235 cm^{-1}; UV (MeOH) λ_{max} (log ε) 241 (2.52) nm; ^1H and ^{13}C NMR (500 MHz for ^1H, C_6D_6) see Table 5; ESIMS *m/z* (relative intensity) 553 ($[M+Na]^+$, 26), 411 (18), 351 (100), 233 (18), 221 (52), 163 (26).

12-Epi-19-deoxyscalarin (42): white amorphous solid; $[\alpha]_D^{25}$ –33.0° (c=0.004, MeOH); IR (thin film) ν_{max} 1765, 1735, 1240 cm^{-1}; UV (MeOH) λ_{max} (log ε) 223 (3.77) nm; ^1H and ^{13}C NMR (500 MHz for ^1H, C_6D_6) see Table 6; ESIMS *m/z* (relative intensity) 429 ($[M+H]^+$, 3), 369 (100), 352 (3), 149 (2).

12-Deacetyl-12-epi-19-deoxyscalarin (43): white needles (CH_3CN); $[\alpha]_D^{25}$ –61.4° (c=0.004, MeOH); IR (thin film) ν_{max} 3450, 1745 cm^{-1}; UV (MeOH) λ_{max} (log ε) 224 (3.72) nm; ^1H and ^{13}C NMR (500 MHz for ^1H, C_6D_6) see Table 6; ESIMS *m/z* (relative intensity) 387 ($[M+H]^+$, 100).

***E*-Neomanoalide diacetate (44):** vicous colorless liquid; $[\alpha]_D^{25}$ –32.9° (c=0.023, MeOH); IR (thin film) ν_{max} 1755, 1225 cm^{-1}; UV (MeOH) λ_{max} (log ε) 224 (3.72) nm; CD (c=4.73×10^{-4} M, MeOH) θ (nm) –5504 (218), –6299 (200); ^1H and ^{13}C NMR (500 MHz for ^1H, C_6D_6) see Table 7; ESIMS *m/z* (relative intensity) 509 ($[M+Na]^+$, 100), 172 (24); HRESIMS *m/z* 509.2898 [$C_{29}H_{42}O_6Na$ requires 509.2868].

***Z*-Neomanoalide diacetate (45):** vicous colorless liquid; $[\alpha]_D^{25}$ –23.3° (c=0.015, MeOH); IR (thin film) ν_{max} 1755, 1225 cm^{-1}; UV (MeOH) λ_{max} (log ε) 224 (3.72) nm; CD (c=3.04×10^{-4} M, MeOH) θ (nm) 0 (271), –10485 (214), –9760 (204); ^1H and ^{13}C NMR (500 MHz for ^1H, C_6D_6) see Table 7; ESIMS *m/z* (relative intensity) 509 ($[M+Na]^+$, 100), 172 (24); HRESIMS *m/z* 509.2794 [$C_{29}H_{42}O_6Na$ requires 509.2868].

Manoalide-25-acetate (46): white needles (*i*-PrOH:C_6H_{14} = 1:5); $[\alpha]_D^{25}$ +25.0° (c=0.004, MeOH); IR (thin film) ν_{max} 3430, 1790, 1770, 1235 cm^{-1}; UV (MeOH) λ_{max} (log ε) 224 (3.81) nm; CD (c=9.60×10^{-5} M, MeOH) θ (nm) 0 (219), –20000 (200); ^1H and ^{13}C NMR (500 MHz for ^1H, C_6D_6) see Table 6; ESIMS *m/z* (relative intensity) 481 ($[M+Na]^+$, 100).

CHAPTER 3

RESULTS AND DISCUSSION

As part of a research project aiming toward a search for chemotherapeutic agents from Thai marine invertebrates, we found that the preliminary screening of the methanolic extract from a Thai sponge, later identified as *Brachiaster* sp., exhibited potent antituberculosis activity (MIC 12.5 μg/mL). This result led to the initiation of a research project in search of active components responsible for such activity. The bioassay-monitored fractionation of the sponge yielded eight sesterterpenes, among which three were new naturally-occurring compounds. All the isolated sesterterpenes were found active in the antituberculsis assay with the MICs of 1.56-50 μg/mL (3-117 μM).

3.1 Isolation of the antituberculosis compounds from the sponge, *Brachiaster* sp.

The Thai sponge, *Brachiaster* sp., was collected at the depth of 18-20 m from Koh-Tao, Surat Thani, Thailand, in April 2001, and was recollected in April 2002 from the same location. The specimen from the first expedition was extracted with MeOH exhaustively (5×1.5 L) and then partitioned with organic solvents to yield hexane-, CH_2Cl_2- and *n*-BuOH-soluble materials (1.11, 1.64 and 1.32 g, respectively). The active CH_2Cl_2 fraction (MIC 6.25 μg/mL) was fractionated and the major active compound, heteronemin **(18)** was obtained (99

mg). The hexane fraction, which was also active (MIC 6.25 μg/mL), was subjected to the chromatographic isolation and two compounds, heteronemin acetate **(41)** and 12-epi-19-deoxyscalarin **(42)** were obtained (10 and 2 mg, respectively). These three compounds are known scalarane-type sesterterpenes.

The sponge was re-collected during the second excursion mentioned earlier. The lyophilized sponge was subsequently extracted with hexane (3×2 L), CH_2Cl_2 (3×2 L) and MeOH (3×2 L) to yield the extracts weighed 13.49, 1.36 and 26.90 g, respectively. The active hexane extract (MIC 3.125 μg/mL) was chosen for the further isolation of the bioactive compounds. An aliquot of the hexane fraction (4.3 g) was separated by means of chromatographic techniques to afford three new compounds, 12-deacetoxy-scalarin acetate **(40)** (3 mg), (*E*)-neomanoalide diacetate **(44)** (4 mg) and (*Z*)-neomanoalide diacetate **(45)** (7 mg), along with two known compounds, 12-deacetyl-12-epi-19-deoxyscalarin **(43)** (2 mg) and manoalide-25-acetate **(46)** (7 mg). Among these, **40** and **43** are also scalarane-type sesterterpenes, whereas **44**, **45** and **46** are the members of the manoalide family of sesterterpenes.

3.2 The structure elucidation of isolated compound

Due to the combining nature of the isolated compounds, i.e., five sesterterpenes of scalarane family and three sesterterpenes of manoalide family, the structure elucidation session of this report will therefore be addressed separately in two sessions. Here, the new compound(s) of either family are presented first, followed by the discussion regarding the identification of the known ones.

3.2.1 The scalarane-sesterterpenes

3.2.1.1 The structure elucidation of 40

Compound **40** was obtained as a white amorphous compound (3 mg) from the hexane-soluble material of the second-expedition specimen by successive chromatographic techniques using SiO_2 column (EtOAc:acetone:hexane = 20:5:75), followed by reverse phase HPLC (ODS, isocratic 85% aqueous CH_3CN; UV detector, 220 nm).

Compound **40** has a molecular formula of $C_{27}H_{40}O_4$ as established by the ESI mass spectrum, which shows a molecular peak at *m/z* 429 ($[M+H]^+$) (Figure 25), and by its 27 carbon signals observed from the ^{13}C NMR spectrum (Figure 4). The proposed molecular formula was confirmed by the $[M]^+$ peak at *m/z* 428.2910 in the HR-EI mass spectrum (calc for $C_{27}H_{40}O_4$ 428.2927). The proposed molecular formula requires the unsaturation degrees of eight. The ^{13}C NMR spectrum (Figure 4) indicates the presence of two carbonyl carbons and one double bond; therefore, five ring systems are required for **40**. The infrared absorptions at ν 1770 and 1730 cm^{-1} (Figure 26) are consistent with the presence of lactone and carbonyl ester functionalities. The UV spectrum (Figure 27) shows the maximal absorption at λ 227 nm (log ε 3.78).

The ^1H NMR spectrum of **40** (Figure 5) in C_6D_6 (500 MHz) exhibits signals for five aliphatic methyl singlets (δ 0.35, 0.54, 0.70, 0.81 and 0.88), one acetate methyl (δ 1.59), one olefinic proton (δ 6.61) and an acetal proton (δ 6.60), along

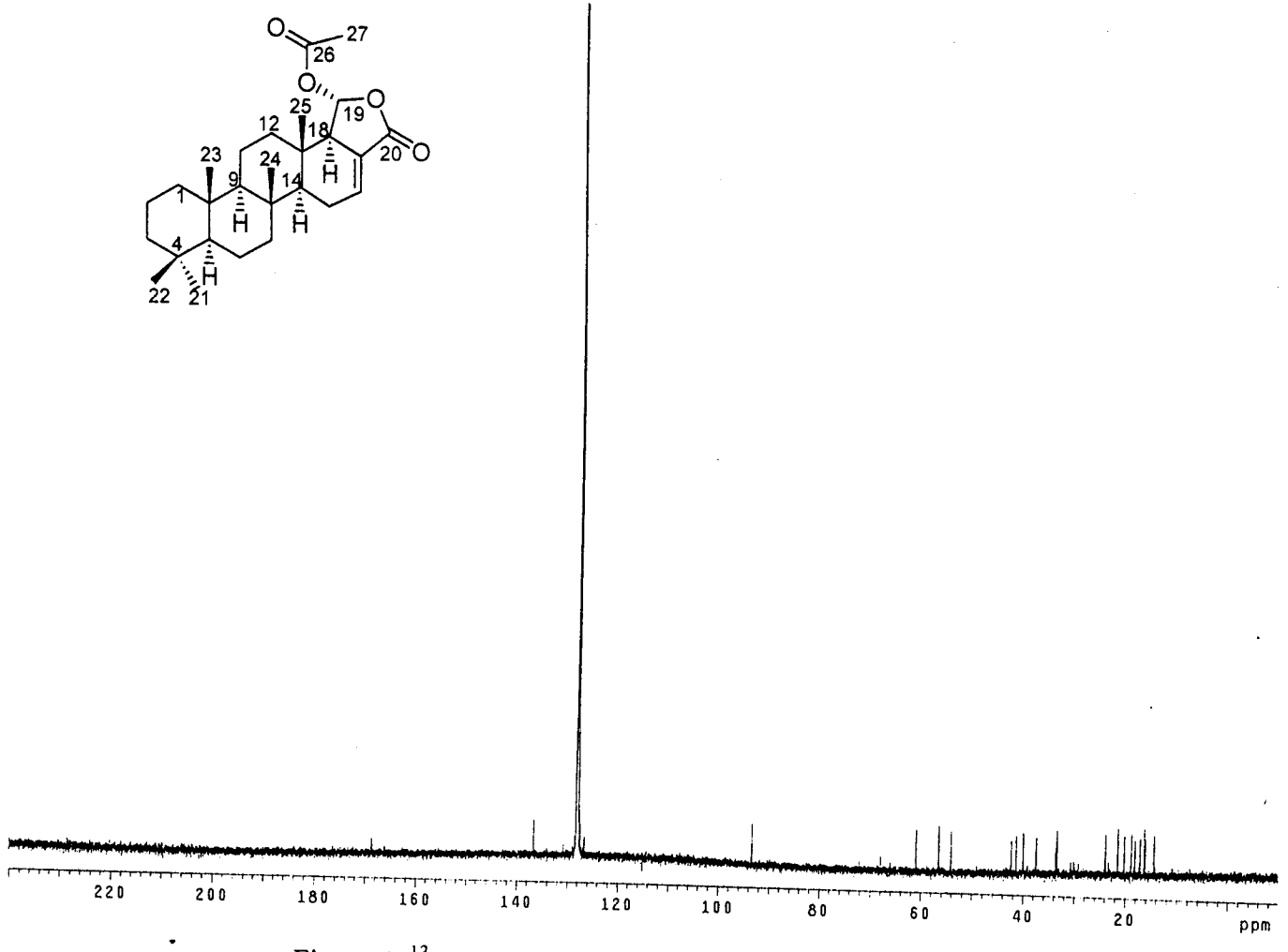


Figure 4 ^{13}C NMR spectrum of **40** (125 MHz; C_6D_6)

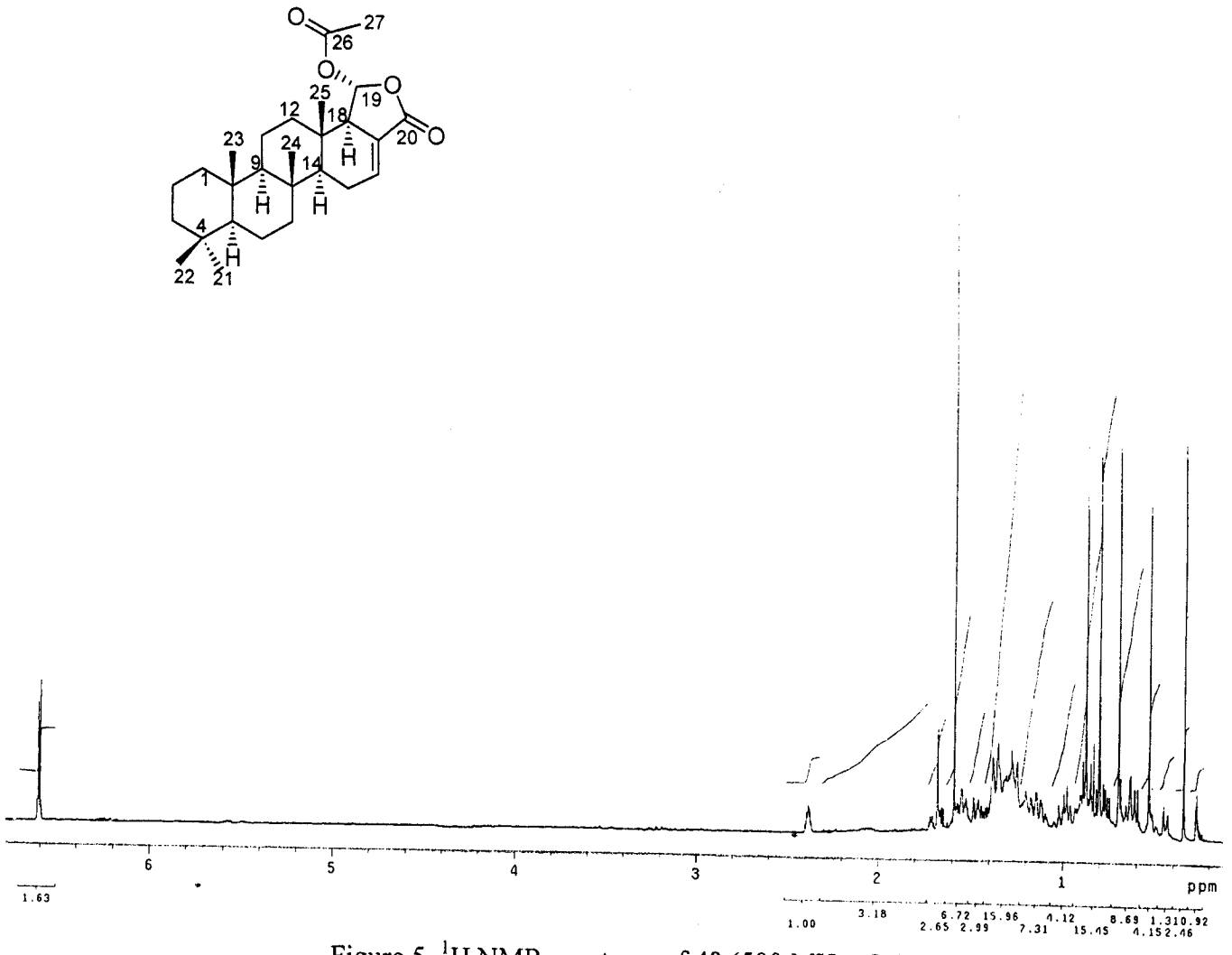


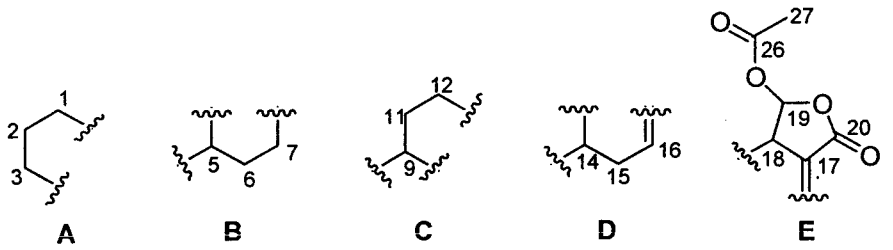
Figure 5 ^{1}H NMR spectrum of **40** (500 MHz; C_6D_6)

with multiplet methylene signals, integrated to belong to sixteen protons. The ^{13}C NMR spectrum (Figure 4) reveals 27 carbons including signals for an α,β-conjugated carbonyl (δ 136.6, 126.6 and 165.7), one ester carbonyl (δ168.6), four quarternary carbons, five methines, eight methylenes, and six methyls, thus accounted for 40 hydrogen atoms.

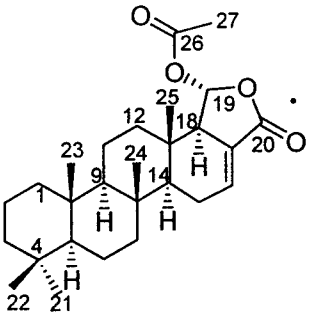
Interpretation of ^{1}H-^{1}H COSY cross peaks in the aliphatic methylene region (Figure 28) led to four partial structures, including fragment **A** [δ 0.64 (m, H-1ax), δ 1.53 (m, H-1eq), δ 1.36 (m, H-2ax), δ 1.55 (m, H-2eq), δ 1.14 (m, H-3ax) and δ 1.37 (m, H-3eq)]; fragment **B** [δ 0.63 (m, H-5), δ 1.15 (m, H-6ax), δ 1.39 (m, H-6eq), δ 0.52 (m, H-7ax) and δ 1.27 (m, H-7eq)]; fragment **C** [δ 0.45 (dd, J = 3.5, 9.6 Hz, H-9), δ 1.00 (m, H-11ax), δ 1.25 (m, H-11eq), δ 0.98 (m, H-12ax) and δ 1.47 (m, H-12eq)]; and fragment **D** [δ 0.78 (dd, J = 5.5, 10.7 Hz, H-14), δ 1.42 (m, H-15a), δ 1.71 (br.d, J = 17.5 Hz, H-15b) and δ 6.61 (ddd, J = 3.6, 4.0. 4.0 Hz, H-16)] as shown below. The assignment of axial and equatorial orientation as stated for each signal was carried out by mean of the coupling constants analysis, along with the observation for the chemical shift of each signal. The axial proton, shielded by 1,3-diaxial repulsion, is generally found at a comparatively higher-field chemical shift than its equatorial counterpart.

The remaining signals, which include those of the acetal proton at δ 6.60 (d, J = 5.5 Hz, H-19) coupling to the methine signal at δ 2.40 (br.ddd, J = 3.6, 3.6, 5.5 Hz, H-18), the signals of lactone carbonyl at δ 165.7 (C-20) and disubstituted

olefinic carbon at δ 126.6 (C-17), all construct the γ-oxygenated γ-lactol moiety. The remaining acetoxy group (δ 168.6, C-26; δ 1.59, s, 3H, H-27) was placed at C-19 on the basis of HMBC data. This structure part is shown as fragment **E**.



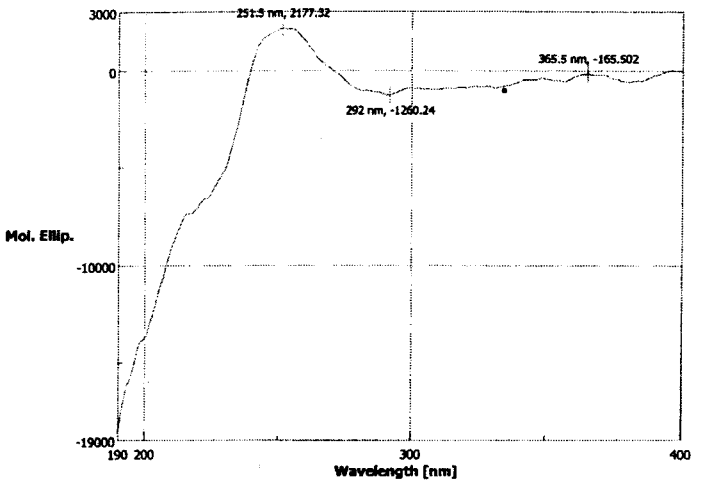
All the proposed structural units were interconnected over four quarternary carbons [δ 33.5 (C-4), 37.4 (C-8), 37.6 (C-10) and 33.7 (C-13)] and five singlet methyls [δ 0.35 (H-25), 0.54 (H-24), 0.70 (H-23), 0.81 (H-21) and 0.88 (H-22)] on the basis of HMBC data (see Table 4). The crucial HMBC correlations include those from C-3 to H-21 and H-22; from C-4 to H-5, H-21 and H-22; from C-5 to H-21, H-22 and H-23; from C-1 to H-9 and H-23; from C-10 to H-5, H-9 and H-23; from C-9 to H-23, H-24, H-5 and H-14; from C-8 to H-9, H-14 and H-24; from C-13 to H-11ax, H-11eq, H-14, H-18 and H-25; and from C-14 to H-24 and H-25. Thus, the tetracarbocyclic moiety (rings A-D) was constructed. The HMBC correlations from C-20 to H-16 and from C-18 to H-14, H-16 and H-25 indicate that the fragment **E** is was fused to ring D at C-17 and C-18. Therefore, the planar structure of **40** was determined as shown. The NMR spectral data are summarized in Table 4.



40

The conformation of tetracarbocyclic ring system of **40** was determined by a close observation of the ^{13}C chemical shifts of ring junction methyls, i.e., C-23, C-24 and C-25 (δ 16.5, 16.1 and 14.5, 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 chair conformation are shielded (thus resonate at < 20 ppm) relative to those on *trans*-fused ring in boat conformation and *cis*-fused ring of both systems. Thus, the conformation of rings A, B and C is assigned as all *trans*. Accordingly, H-5, H-9 and H-14 are assigned as axial.

The stereochemistry of **40** at positions 18 and 19 was assigned on the basis of coupling constant and CD spectral analysis and was comfirmed by nOe observation. Allylic coupling between H-18 and H-16 (J = 3.6 Hz), and homoallylic coupling between H-18 and H-15a (J = 3.6 Hz), suggested that H-18 is pseudoaxial (Pretsch *et al.*, 1989). The CD spectrum of **40** (Figure 6) reveals the first positive Cotton effect ($[\theta]_{251.5}$ +2177), indicating that the configurations at C-13 and C-19 are *S* and *R*, respectively, according to the octant rule (Eliel and Wilen, 1994).



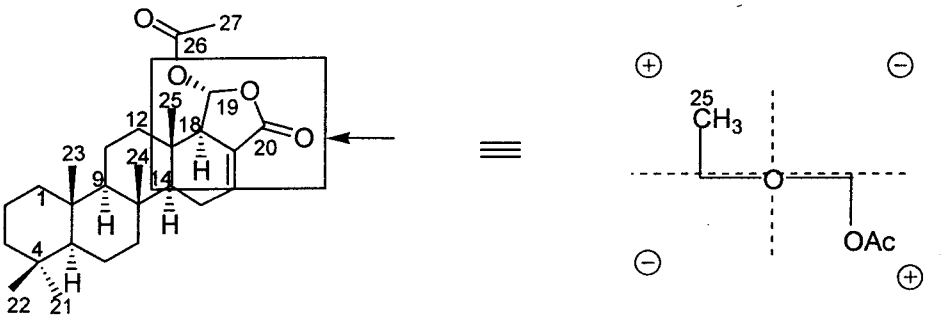


Figure 6 CD spectrum of compound **40**

This is comfirmed by comparison with the report data (Cimino *et al.*, 1977) of the positive cotton effect ($[\theta]_{244}$ +14980) and the $[\alpha]_D$ (-11.5°) of 12-epi-19-deoxyscalarin; i.e., both compounds thus share similar configuration. Furthermore, the dipolar couplings between H-18 and H-14, and between H-19 and H-25 observed from nOe difference spectra (Figures 31 and 32) indicate that the protons of each pair reside on the same plane of structure; thus strongly comfirm the proposed stereochemistry. Compound **40**, therefore, is proposed as 12-deacetoxy-scalarin acetate with the absolute of ring D and E portion configuration shown in figure 7.

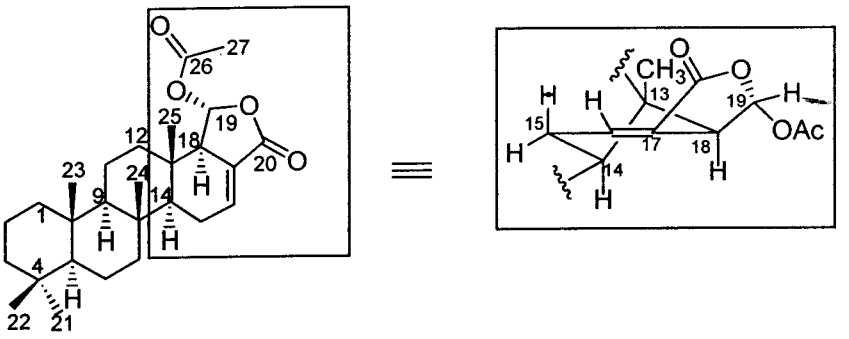


Figure 7 The stereochemistry of **40**

Table 4 NMR data (500 MHz for ^{1}H; in C_6D_6) of **40**

Position	δ_H (mult.; *J* in Hz)	δ_C (mult.)	HMBC correlation ($^{13}C \longrightarrow {}^{1}H$)
1	Hax, 0.64 (m)	40.0 (t)	H-2ax, H-2eq, H-9, H-23
	Heq, 1.53 (m)		
2	Hax, 1.36 (m)	18.9 (t)	H-1ax, H-3ax, H-3eq, H-21
	Heq, 1.55 (m)		
3	Hax, 1.14 (m)	42.4 (t)	H-21, H-22
	Heq, 1.37 (m)		
4	-	33.5 (s)	H-5, H-21, H-22
5	0.63 (m)	56.5 (d)	H-21, H-22, H-23
6	Hax, 1.15 (m)	18.2 (t)	H-5, H-7ax, H-7eq
	Heq, 1.39 (m)		
7	Hax, 0.52 (m)	41.4 (t)	H-5, H-24
	Heq, 1.27 (m)		
8	-	37.4 (s)	H-9, H-14, H-24
9	0.45 (dd, 3.5, 9.6)	61.0 (d)	H-5, H-14, H-23, H-24
10	-	37.6 (s)	H-5, H-9, H-24

Table 4 (cont.)

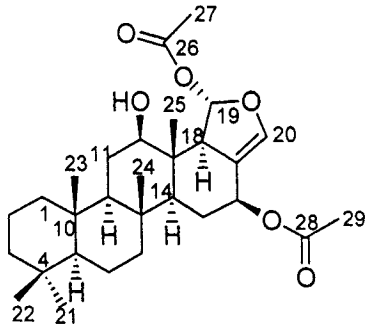
Position	δ_H (mult.; *J* in Hz)	δ_C (mult.)	HMBC correlation ($^{13}C \longrightarrow {}^{1}H$)
11	Hax, 1.00 (m) Heq, 1.25 (m)	17.1 (t)	H-9, H-12ax, H-12eq
12	Hax, 0.98 (m) Heq, 1.47 (m)	40.0 (t)	H-9, H-25
13	-	33.7 (s)	H-11ax, H-11eq, H-12eq, H-14, H-18, H-25
14	0.78 (dd, 5.5, 10.7)	54.2 (d)	H-24, H-25
15	Ha, 1.42 (m) Hb, 1.71 (br.d, 17.5)	23.9 (t)	H-14
16	6.61 (br.ddd, 3.6, 4.0, 4.0)	136.6 (d)	-
17	-	126.6 (s)	-
18	2.40 (br.ddd, 3.6, 3.6, 5.5)	56.6 (d)	H-14, H-16, H-25
19	6.60 (d, 5.5)	93.4 (d)	H-27
20	-	165.7 (s)	H-16, H-19
21	0.81 (s, 3H)	21.4 (q)	H-22
22	0.88 (s, 3H)	33.3 (q)	H-5, H-21
23	0.70 (s, 3H)	16.5 (q)	H-5, H-9
24	0.54 (s, 3H)	16.1 (q)	H-9, H-14
25	0.35 (s, 3H)	14.5 (q)	H-14
26	-	168.6 (s)	H-19, H-27
27	1.59 (s, 3H)	20.2 (q)	-

3.2.1.2 The structure elucidation of 18

Compound **18**, which is the major component, is obtained as white needles (99 mg) from the CH_2Cl_2-soluble material of the first expedition using chromatographic technique with Sephadex LH-20 (methanol) then SiO_2 columns (3% EtOAc in CH_2Cl_2). Also, the hexane-soluble material of second expedition specimen was isolated by chromatographic technique using SiO_2 columns (20:5:75 of EtOAc:acetone: hexane) and re-crystallized in CH_2Cl_2-methanol mixture (1:3) to afford **18** (390 mg).

The ESI mass spectrum of **18** exhibits a molecular ion peak at *m/z* 511 ($[M+Na]^+$) (Figure 33). Along with 29 carbon signals observed from its ^{13}C NMR spectrum (Figure 8), this corresponds with the molecular formula of $C_{29}H_{44}O_6$. Accordingly, the eight degrees of unsaturation are determined as two carbonyl carbons, two sp^2 carbons and five ring systems. The major absorption band at ν 3500 cm^{-1} was assigned to the hydroxyl group, whereas those at 1740 and 1235 cm^{-1} were assigned to the ester functionality in the IR spectrum (Figure 34). The UV spectrum (Figure 35) shows the absorption maximum at λ 229 nm (log ε 2.34).

The ^1H NMR spectrum of **18** (Figure 9) displays signals of five singlet aliphatic methyls, two acetate methyls, seven methylenes, seven methines (which include one acetal proton and two oxygenated methine protons), and one olefinic proton. The 29 carbon signals in the ^{13}C NMR spectrum (Figure 8) indicate the presence of seven methyls, seven methylenes, one acetal methine, two carbonol



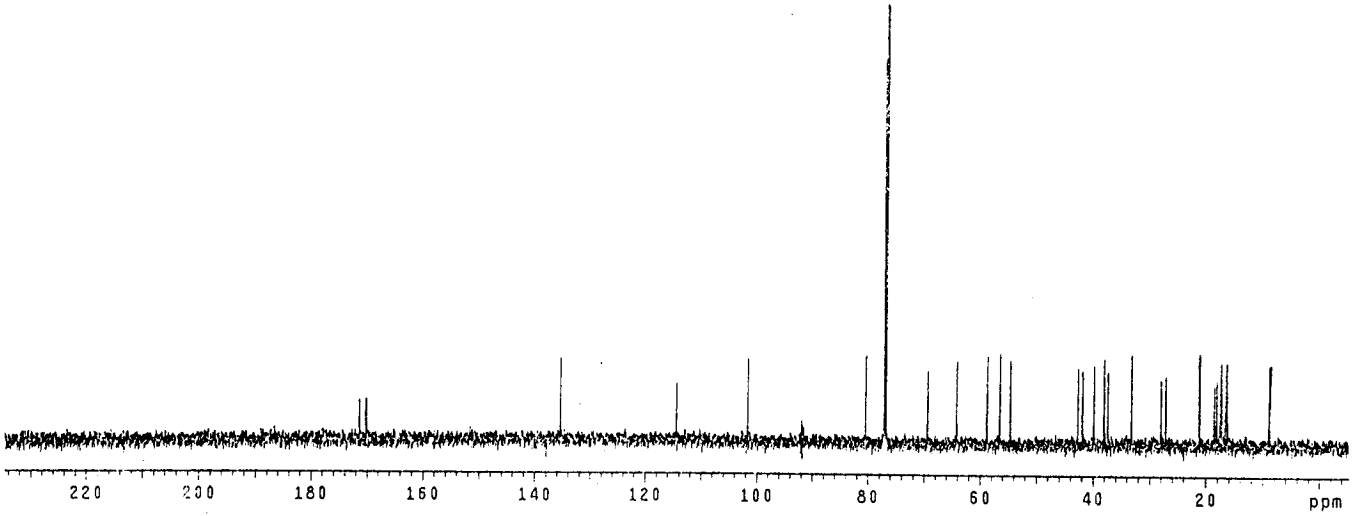


Figure 8 ^{13}C NMR spectrum of **18** (125 MHz; $CDCl_3$)

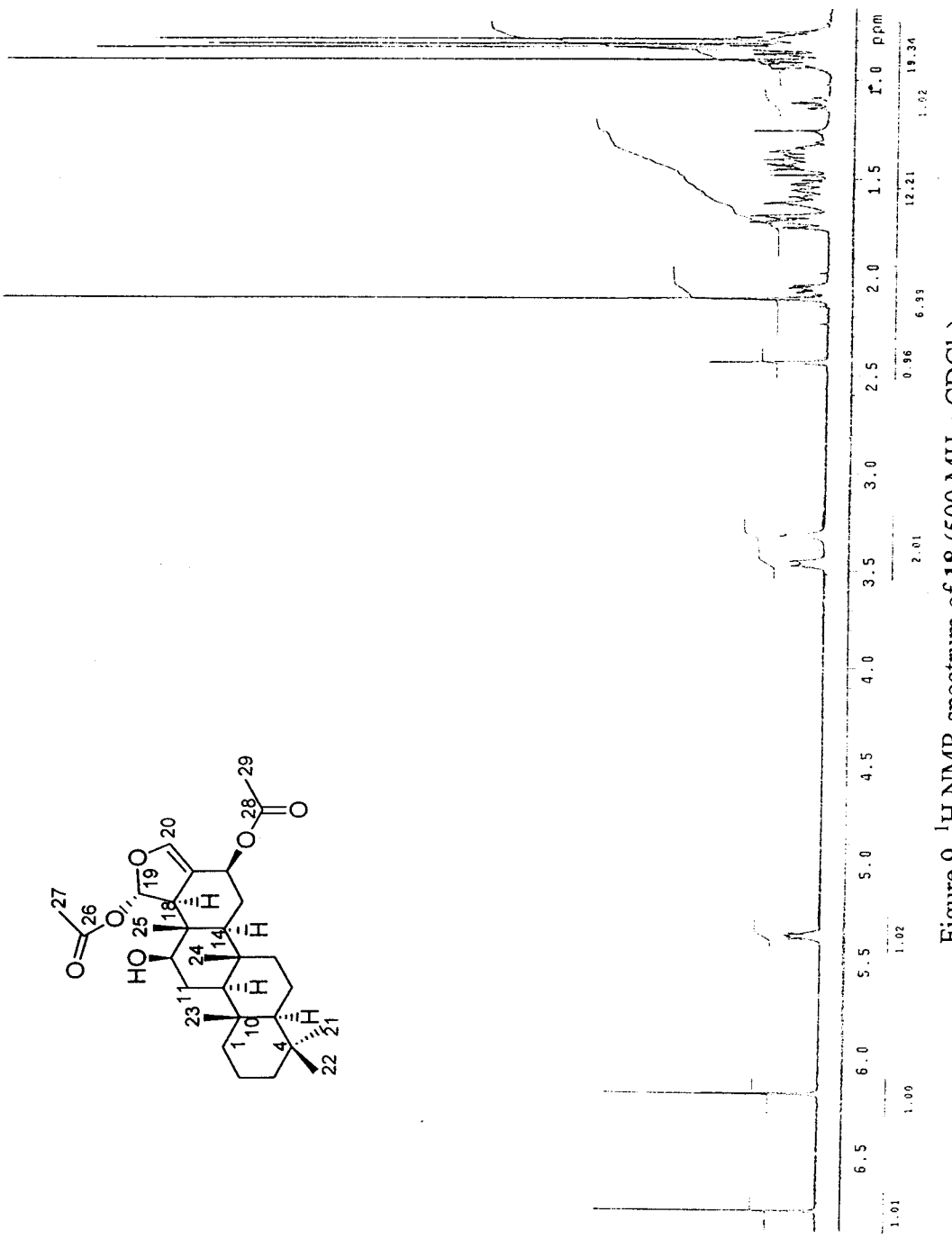


Figure 9 ^{1}H NMR spectrum of **18** (500 MHz; $CDCl_3$)

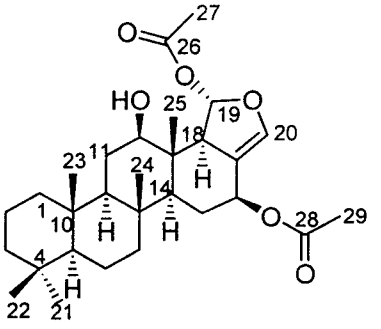
methines, four aliphatic methines, four sp^3 quarternary carbons, two olefinic carbons and two carbonyls. Interpretation of the ^1H-^1H COSY and HMQC spectra (Figures 36 and 37) led to the assembly of the partial structures of C-1 to C-3 and C-5 to C-7 similar to those seen in **40**. The subunit of C-9 to C-12 and C-14 to C-16 were established in the same fashion, however, with some slight differences in oxygenating patterns on C-12 and C-16. Here, a hydroxy group was proposed to attach on C-12 due to the chemical shift at 3.45 ppm (br.d, J = 11.4 Hz, H-12), whereas an acetoxy group (δ_C 170.1, C-28; δ_H 2.11, s, 3H, H-29) was linked to C-16 as a signal at δ 5.37 (dddd, J = 1.8, 1.8, 6.1, 10.4 Hz, H-16) was observed.

The remaining acetal proton at δ 6.78 (d, J = 1.4 Hz, H-19) is coupled to a methine proton at δ 2.43 (br.s, H-18), which shows further allylic coupling to an olefinic proton at δ 6.17 (t, J = 1.8 Hz, H-20). Also, by the C-H long range correlations, the acetoxy moiety with the carbonyl at δ 171.3 (C-26) and the methyl at δ 2.11 (s, 3H, H-27) was placed onto C-19. This information suggests the presence of a dihydrofuranol acetate moiety, which fuses to the C-17-C-18 bond.

Connectivities of all the proposed partial structures were deduced by the HMBC analysis (Figure 38) and led to the proposed structure of **18** as shown. The all *trans* fused ring system was demonstrated based on the ^{13}C NMR chemical shifts of the axial methyl groups including signals of C-23 (δ 16.3), C-24 (δ 17.3) and C-25 (δ 8.7). Here, all the methyl groups, except for C-22, are resonating at high field region with the chemical shifts less than 25 ppm, characteristic to the axial methyl shielded by the 1,3-diaxial effect.

The stereochemistry at positions 12, 16, 18 and 19 of **18** was determined by the observations of chemical shifts and coupling constants. H-12 and H-16 are axial, as established from their large coupling constants (J = 11.4 and 10.4 Hz, respectively). Extended to the nearby position 18, C-25 shift was used as an indirect determination of the relative stereochemistry at C-12 and C-18 in the scalarane series. (Cimino *et al.*, 1977; Crew and Bescansa, 1986). It is the additive γ-effect that induces the up-field shift of C-25 signals in those of with 12β and 18β skeleton. The chemical shift of C-25 in **18** (δ 8.7), similar to that found in 12β,18β-substituted scalaranes, indicates that H-12 and H-18 are axial.

Similar to **40**, the relative stereochemisty between H-18 and H-19 is *anti*, as determined by the small coupling constant (J = 1.4 Hz) (Crews and Bescansa, 1986). The similar sign in the specific rotation ($[\alpha]_D^{25}$ –71.4°) to those previously report (Bourquet-Kondracki *et al.*,1994) indicates the same configuration; thus the structure of **18** is shown below.



18

By comparison of the ^1H and ^{13}C spectral data of **18** with the previously reported data (Kazlauskas *et al.*, 1976; Crews and Bescansa, 1986), compound **18**

was identical with heteronemin, which was first isolated from the sponge *Heteronema erecta* (Kazlauskas *et al.*, 1976). The NMR spectral data of **18** are shown in Table 5.

3.2.1.3 The structure elucidation of 41

Compound **41** was isolated as white amorphous solid (10 mg). It was purified from hexane-soluble material of the first expedition specimen using schromatographic techniques with SiO_2 column (5% EtOAc in CH_2Cl_2) then reverse phase HPLC (ODS, isocratic 87% aqueous CH_3CN; UV detector, 220 nm).

The molecular formula of compound **41** was deduced to be $C_{31}H_{46}O_7$ by means of the analyses of its ESI mass spectrum (Figure 39), which shows molecular ion peak at *m/z* 553 ($[M+Na]^+$), as well as its and ^{13}C NMR spectrum (Figure 10). The nine-degree of unsaturation is designated as three carbonyl functionalities, one carbon-carbon double bond and five ring systems. The IR spectrum of **41** (Figure 40) shows the bands at ν 1740 and 1235 cm^{-1}, which were assigned to the ester carbonyl functionality, while the UV spectrum (Figure 41) shows the absorption maximum at λ 241 nm (log ε 2.52).

Despite the difference operating NMR solvents, the ^{13}C NMR spectrum of **41** (in C_6D_6) and **18** (in $CDCl_3$) were almost identical, suggesting that both shared a common skeleton. However, the ^1H NMR spectrum of **41** (Figure 11) obtained from C_6D_6 exhibits several protons that resonates at higher field than those of **18** ($CDCl_3$). The up-field shifts, particularly of the protons residing in the proximity

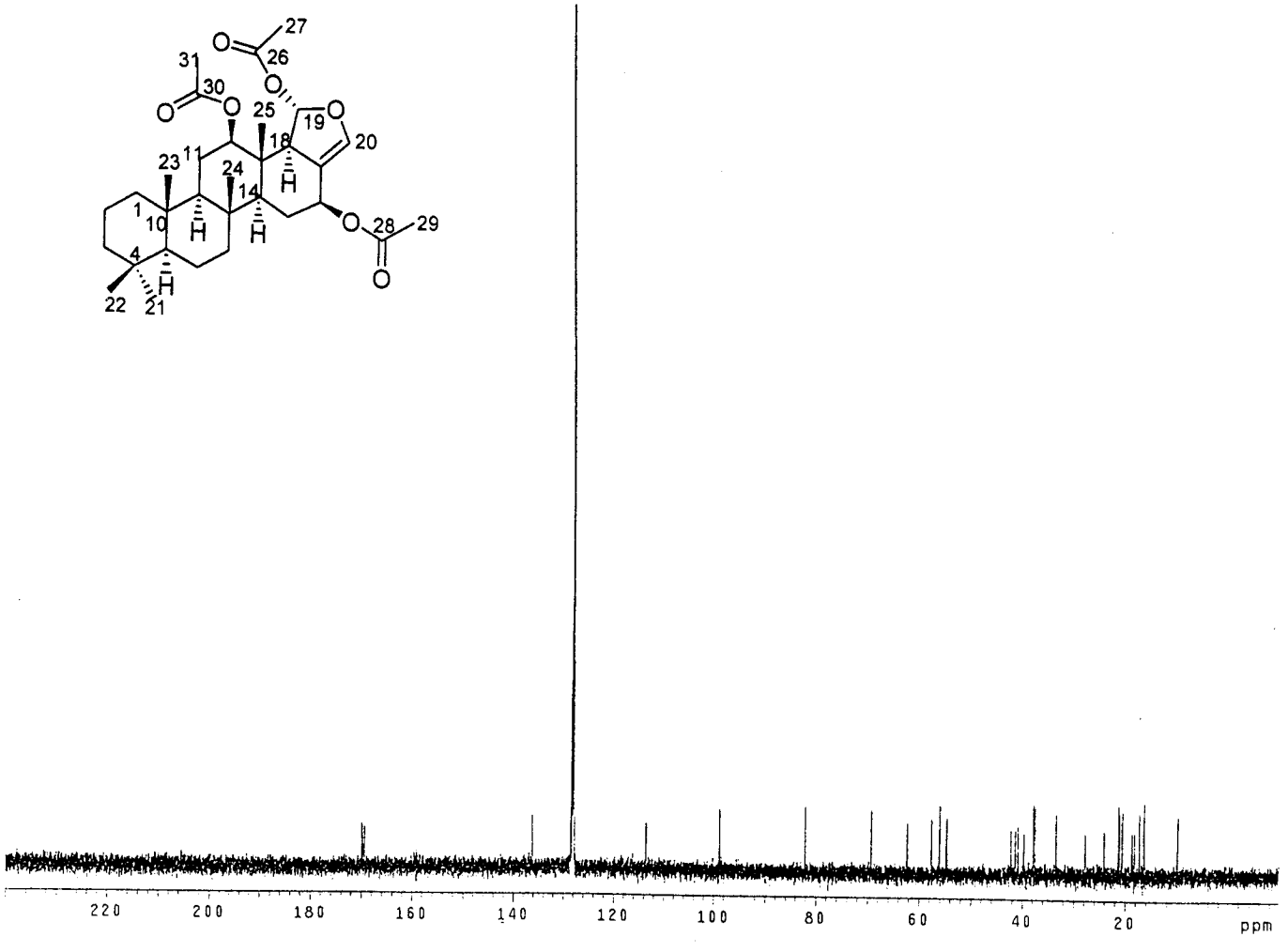


Figure 10 ^{13}C NMR spectrum of **41** (125 MHz; C_6D_6)

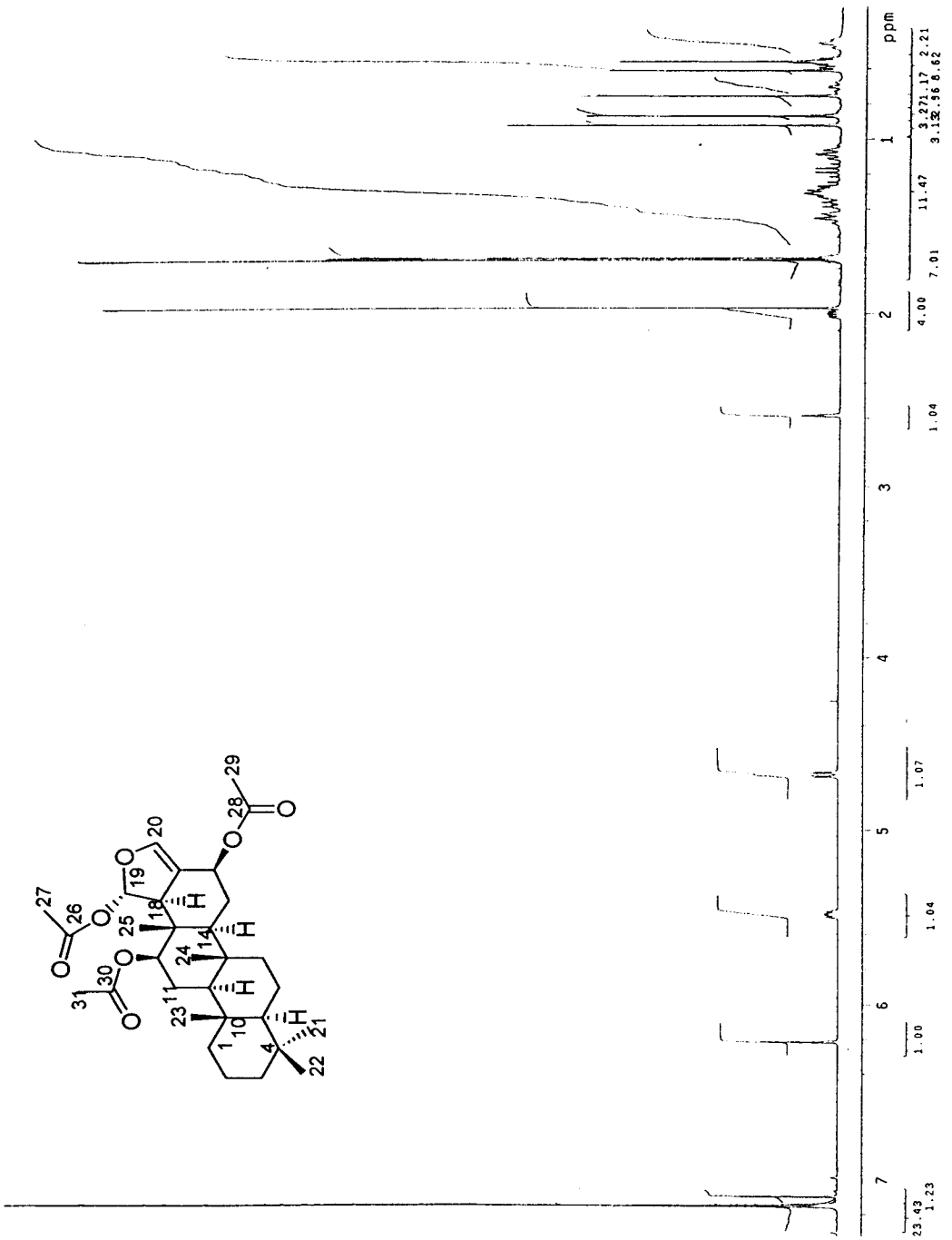
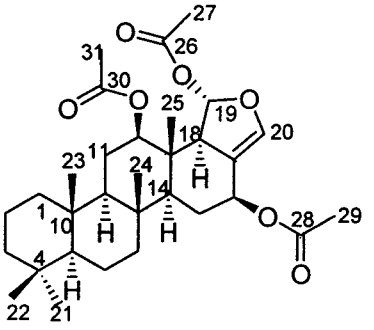


Figure 11 ^{1}H NMR spectrum of **41** (500 MHz; C_6D_6)

of the carbonyls, are resulted from the diamagnetic anisotropy caused by the complex between the carbonyl groups and solvent benzene (Williams and Bhacca, 1965).

Apart form the up-field shifts caused by the solvent effect stated above, the major differences between the ^{1}H spectra of **41** and **18** are the otherwise down-field signal at δ 4.68 (dd, J = 4.2, 11.4 Hz, H-12), along with the additional acetoxy signals at δ 169.9 (C-30), 21.1 (C-31) and 1.97 (s, 3H, H-31). This clearly indicates that **41** in fact is an acetate analog of **18**, of which the acetate group was added onto 12-OH group. The large coupling constant (J = 11.4 Hz) observed for H-12 of **41** indicates the axial orientation similar to that of **18**.

Therefore, compound **41** was identified as heteronemin acetate. The NMR spectral data of **41** are consistent with the data for previously reported from the sponge *Hyrtios erecta*, furthermore, the stereochemistry of **41** was confirmed by the specific rotation ($[\alpha]_D^{25}$ –48.6°), which is in the same sign as those report ($[\alpha]_D$ –30°) (Crews and Bescansa, 1986). The NMR spectral data of **41** are summarized in Table 5.



41

Table 5 NMR data (500 MHz for ^1H) of **18** (in $CDCl_3$) and **41** (in C_6D_6)

Position	δ_H (mult.; *J* in Hz)		δ_C (mult.)	
	18	**41**	**18**	**41**
1	Hax, 0.78 (m)	Hax, 0.69 (m)	39.9 (t)	39.7 (t)
	Heq, 1.69 (m)	Heq, 1.45 (m)		
2	Hax, 1.34 (m)	Hax, 1.48 (m)	18.2 (t)	18.3 (t)
	Heq, 1.54 (m)	Heq, 1.54 (m)		
3	Hax, 1.12 (m)	Hax, 1.09 (m)	42.0 (t)	41.4 (t)
	Heq, 1.37 (m)	Heq, 1.32 (m)		
4	-	-	33.2 (s)	33.4 (s)
5	0.77 (m)	0.58 (m)	56.5 (d)	56.1 (d)
6	Hax, 1.42 (m)	Hax, 1.06 (m)	18.6 (t)	18.7 (t)
	Heq, 1.62 (m)	Heq, 1.31 (m)		
7	Hax, 0.91 (m)	Hax, 0.47 (m)	41.8 (t)	40.8 (t)
	Heq, 1.74 (m)	Heq, 1.39 (ddd, 3.2, 3.2 ,12.7)		
8	-	-	37.4 (s)	37.5 (s)
9	0.86 (m)	0.56 (m)	58.7 (d)	57.6 (d)
10	-	-	38.0 (s)	37.8 (s)
11	Hax, 1.46 (m)	Hax, 1.18 (m)	27.2 (t)	24.1 (t)
	Heq, 1.71 (m)	Heq, 1.70 (m)		
12	3.45 (br.d, 11.4)	4.68 (dd, 4.2, 11.4)	80.5 (d)	82.1 (d)
13	-	-	42.7 (s)	42.2 (s)
14	0.94 (m)	0.59 (m)	54.6 (d)	54.7 (d)
15	Hax, 1.41 (m)	Hax, 1.29 (m)	28.0 (t)	28.0 (t)
	Heq, 2.06 (ddd, 2.4, 6.1, 12.1)	Heq, 2.01 (ddd, 2.2, 6.0, 12.0)		
16	5.37 (dddd, 1.8, 1.8, 6.1, 10.4)	5.47 (dddd, 1.9, 1.9, 6.0, 10.4)	69.3 (d)	69.3 (d)
17	-	-	114.4 (s)	113.6 (s)
18	2.43 (br.s)	2.58 (br.s)	64.2 (d)	62.3 (d)

Table 5 (cont.)

Position	δ_H (mult.; *J* in Hz)		δ_C (mult.)	
	18	**41**	**18**	**41**
19	6.78 (d, 1.4)	7.10 (d, 2.2)	101.7 (d)	98.8 (d)
20	6.17 (t, 1.8)	6.21 (t, 1.9)	135.3 (d)	136.2 (s)
21	0.79 (s, 3H)	0.75 (s, 3H)	21.4 (q)	21.3 (q)
22	0.83 (s, 3H)	0.87 (s, 3H)	33.2 (q)	33.3 (q)
23	0.82 (s, 3H)	0.61 (s, 3H)	16.3 (q)	16.4 (q)
24	0.84 (s, 3H)	0.56 (s, 3H)	17.3 (q)	17.3 (q)
25	0.90 (s, 3H)	0.92 (s, 3H)	8.7 (q)	10.0 (q)
26	-	-	171.3 (s)	169.7 (s)
27	2.11 (s, 3H)	1.69 (s, 3H)	21.2 (q)	20.5 (q)
28	-	-	170.1 (s)	169.3 (s)
29	2.11 (s, 3H)	1.67 (s, 3H)	21.1 (q)	20.5 (q)
30	-	-	-	169.9 (s)
31	-	1.97 (s, 3H)	-	21.3 (q)
12-OH	3.32 (d, 4.7)	-	-	-

3.2.1.4 The structure elucidation of 42

As white amorphous solid (2 mg), compound **42** was obtained from hexane-soluble material of the first expedition specimen within the same step as that for **41**, i.e., SiO_2 column (5% EtOAc in CH_2Cl_2) then reverse phase HPLC (ODS, isocratic 87% aqueous CH_3CN; UV detector, 220 nm).

Similar to that of **40**, compound **42** has the molecular formula of $C_{27}H_{40}O_4$, as deduced from its molecular ion peak at *m/z* 429 ($[M+H]^+$) in the ESI mass

spectrum (Figure 45), and from the numbers of carbons and protons observed from the ^{13}C and ^{1}H spectra (Figures 12 and 13). Also similar to **40**, the nine-degree of unsaturation is assigned as two carbonyls, one carbon-carbon double bonds and five ring systems. The lactone and ester functionalities was determined from the major absorption bands at ν 1765, 1735 and 1240 cm^{-1} in its IR spectrum (Figure 46). The UV maximal absorption of **42** (Figure 47) was observed at λ 223 nm (log ε 3.77).

Despite similar NMR spectral pattern, thus suggesting **18**, **40** and **42** share the same skeleton, close inspection indicates that the NMR signals associated with the acetal moiety as seen in **18** and **40** are absent here. On the other hand, compound **42** shows two characteristic lactone methylene signals at δ 3.82 (dd, J = 9.2, 9.3 Hz, H-19a) and 3.92 (dd, J = 9.1, 9.2 Hz, H-19b), corresponding to the carbon at δ 67.2 (C-19). These indicate the presence of a saturated γ-lactone, replacing a lactol moiety seen previously in **18** and **40**. Additionally, an acetoxy group (δ_C 169.3, C-26; δ_H 1.54, s, 3H, H-27) was proposed to attached on C-12 due to the chemical shift at δ 4.46 (dd, J = 4.3, 11.5 Hz, H-12). Here, **42** is proposed to be a 12-acetoxy analog of the scalarin family with an extended saturated lactone ring E. This proposed structure is identical to the known sesterterpene, 12-epi-19-deoxyscalarin, as shown.

The coupling constant of 11.5 Hz observed for H-12 indicates the axial orientation similar to that of **18**. Also, the orientation of H-18 of **42** is pseudoaxial, similar to that of **18** and **40**, due to the allylic coupling constant (3.6 Hz) between

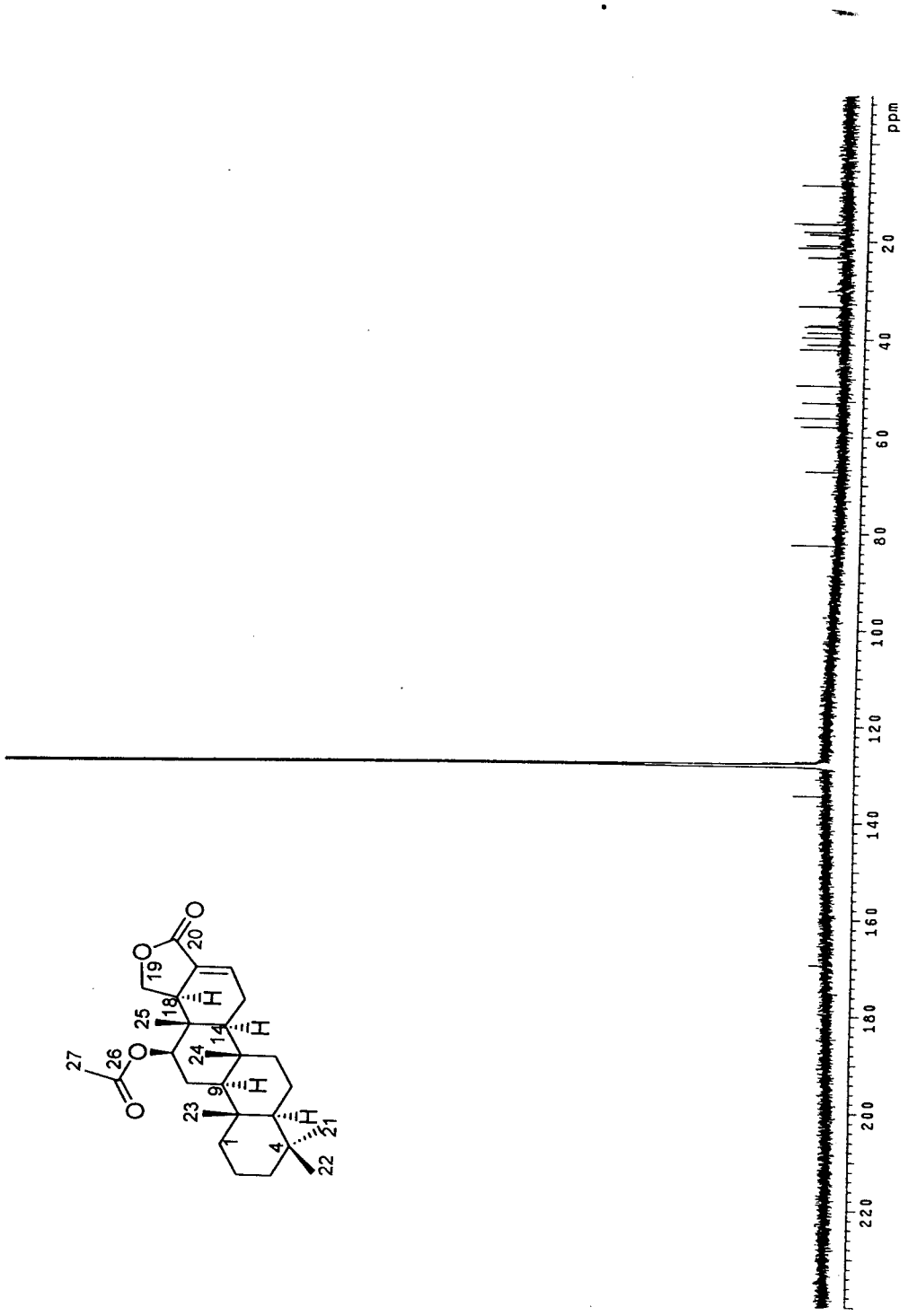


Figure 12 ^{13}C NMR spectrum of **42** (125 MHz; C_6D_6)

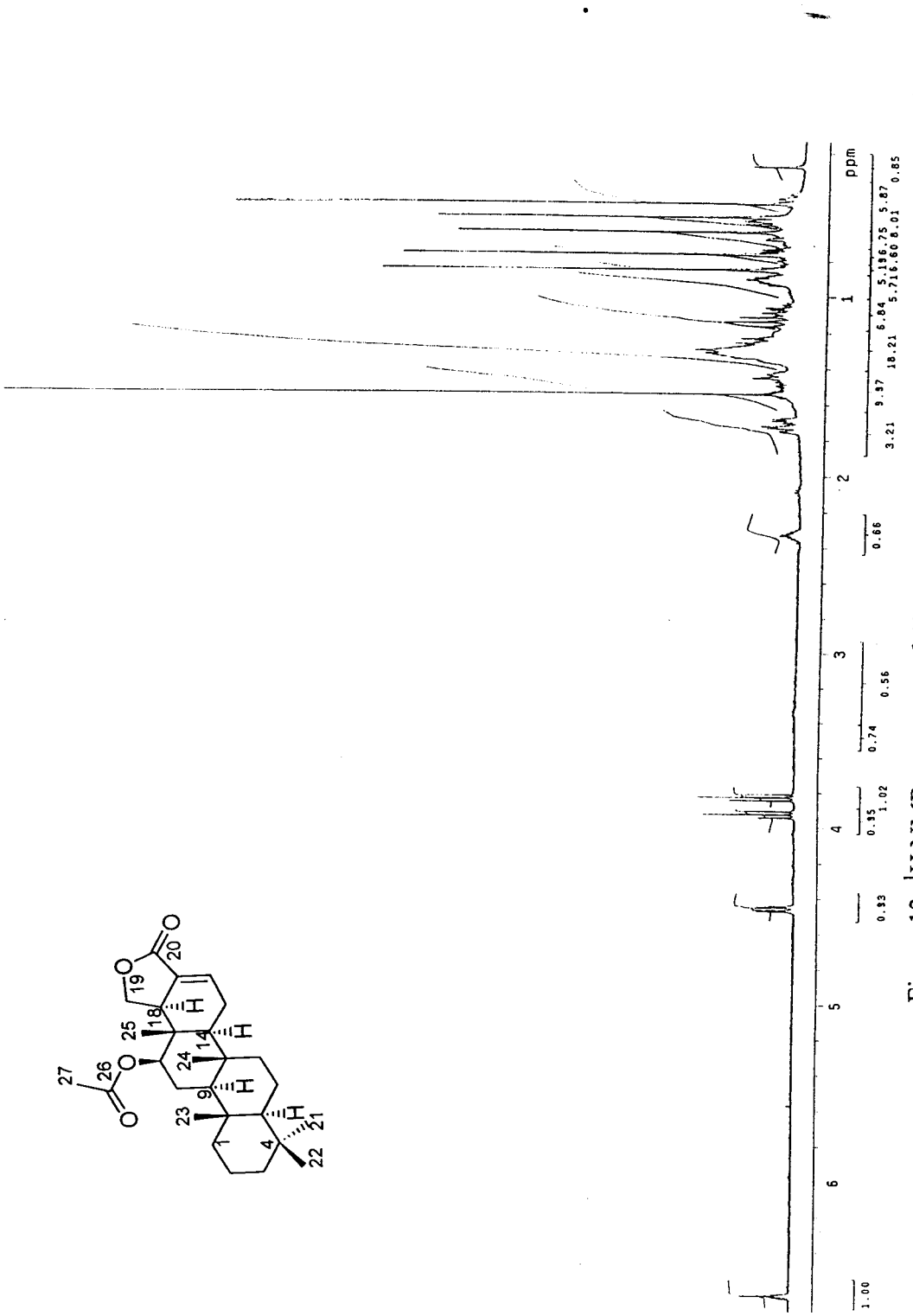
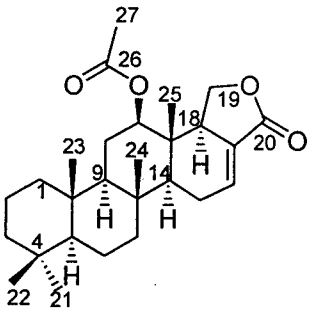


Figure 13 ^{1}H NMR spectrum of **42** (500 MHz; C_6D_6)

H-18 and H-16, and the homoallylic coupling constant (3.9 Hz) between H-18 and H-15a (Pretsch *et al.*, 1989).

Comparison between ^{1}H and ^{13}C NMR data and specific rotation ($[\alpha]_D^{25}$ –33.0°) of **42** with those previously reported strongly supports the proposed structure (Cimino *et al.*, 1977). Therefore, compound **42** was identified as 12-epi-19-deoxyscalarin. The NMR spectral data of **42** are summarized in Table 6.



42

3.1.2.5 The structure elucidation of 43

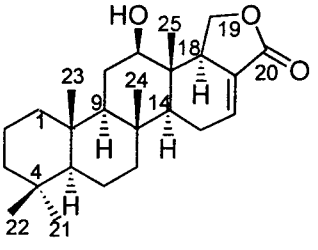
Compound **43** was isolated as white needles (2 mg) from hexane-soluble material of the second-expedition specimen by successive chromatographic techniques using SiO_2 column (EtOAc:acetone:hexane = 20:5:75) followed by semi-preparative normal phase HPLC (SiO_2, isocratic 5% isopropanol in hexane; UV detector, 220 nm) and re-crystallized in acetonitrile.

The molecular formula of $C_{25}H_{38}O_3$ was determined by means of the ESI mass spectral analysis (Figure 51), which show molecular ion peak at *m/z* 387 ($[M+H]^+$). The ^{13}C NMR spectrum (Figure 14) indicates the presence of one

carbonyl carbons and one double bond, thus, five ring systems are required to fit the unsaturation degree of seven. The IR spectrum (Figure 52) shows absorption bands at ν 3450 and 1745 cm^{-1}, which were assigned to hydroxyl and lactone functionalities, respectively. The UV spectrum (Figure 53) shows the absorption maximum at λ 224 nm (log ε 3.72).

The ^{13}C and ^{1}H NMR spectra of **43** (Figures 14 and 15) were almost identical to those of **42**. The only difference is the absence of the acetyl group at C-12. The up-field shift of H-12 (δ 2.72, ddd, J = 4.5, 5.0, 11.4 Hz) indicates that **43** is a deacetyl analog of **42**. The orientation of H-12 is assigned as axial, similar to **18**, **41** and **42**, as determined by the coupling constant of 11.4 Hz. Interestingly, the chemical shift of H-12 at 2.72 ppm is uncharacteristically high-fielded, presumably due to diamagnetic anisotropy from solvent effect (Williams and Bhacca, 1965).

Compound **43** was, therefore, identified as 12-deacetyl-12-epi-19-deoxyscalarin. The substitution pattern of **43** was confirmed by comparison with the spectral data of those previously reported (Cimino *et al.*, 1977). Although its specific rotation has never been reported, the similar sign to that of **42**, suggests the two compounds share the same configuration. The NMR spectral data of **43** are summarized in Table 6.



43

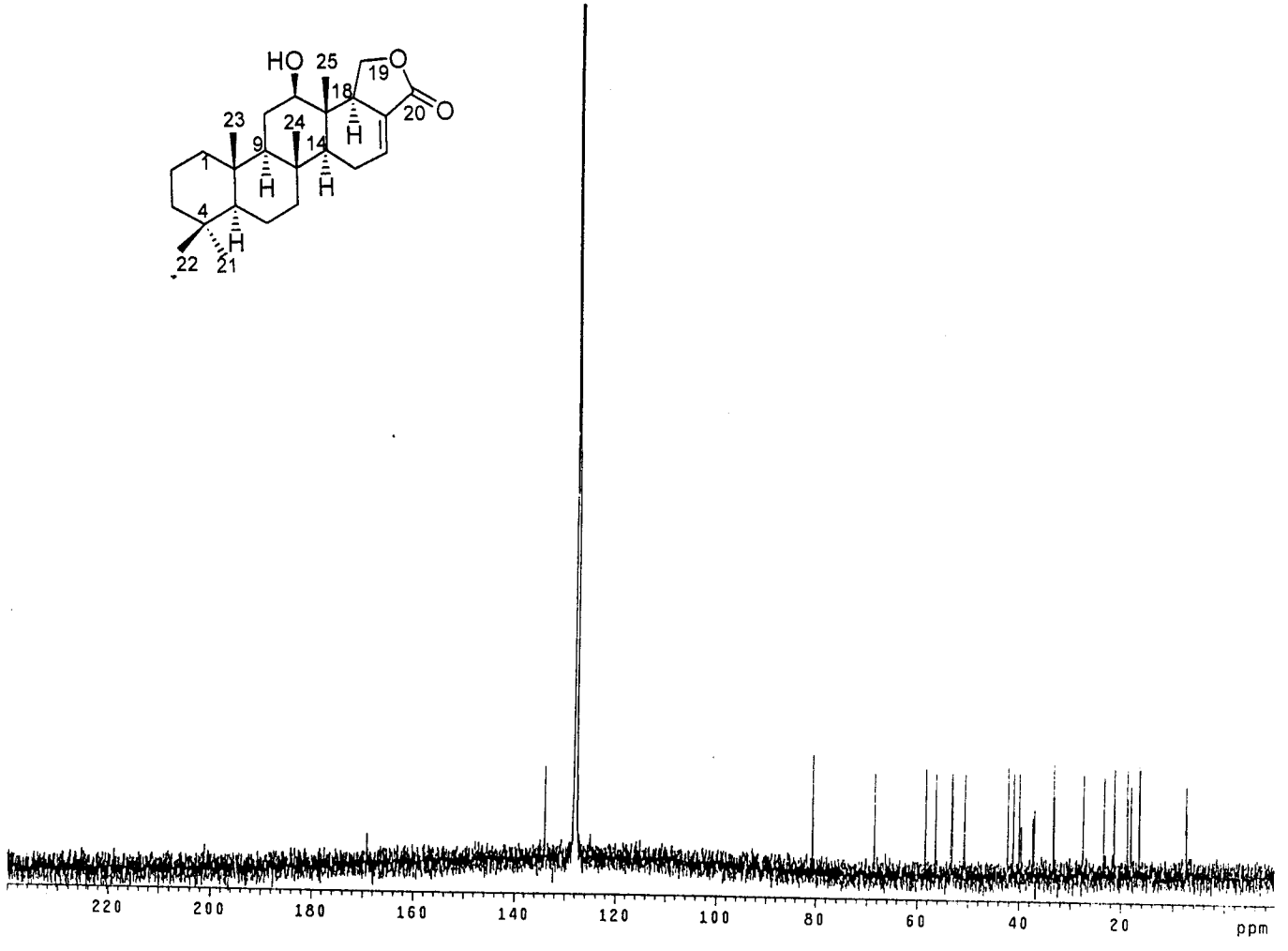


Figure 14 ^{13}C NMR spectrum of **43** (125 MHz; C_6D_6)

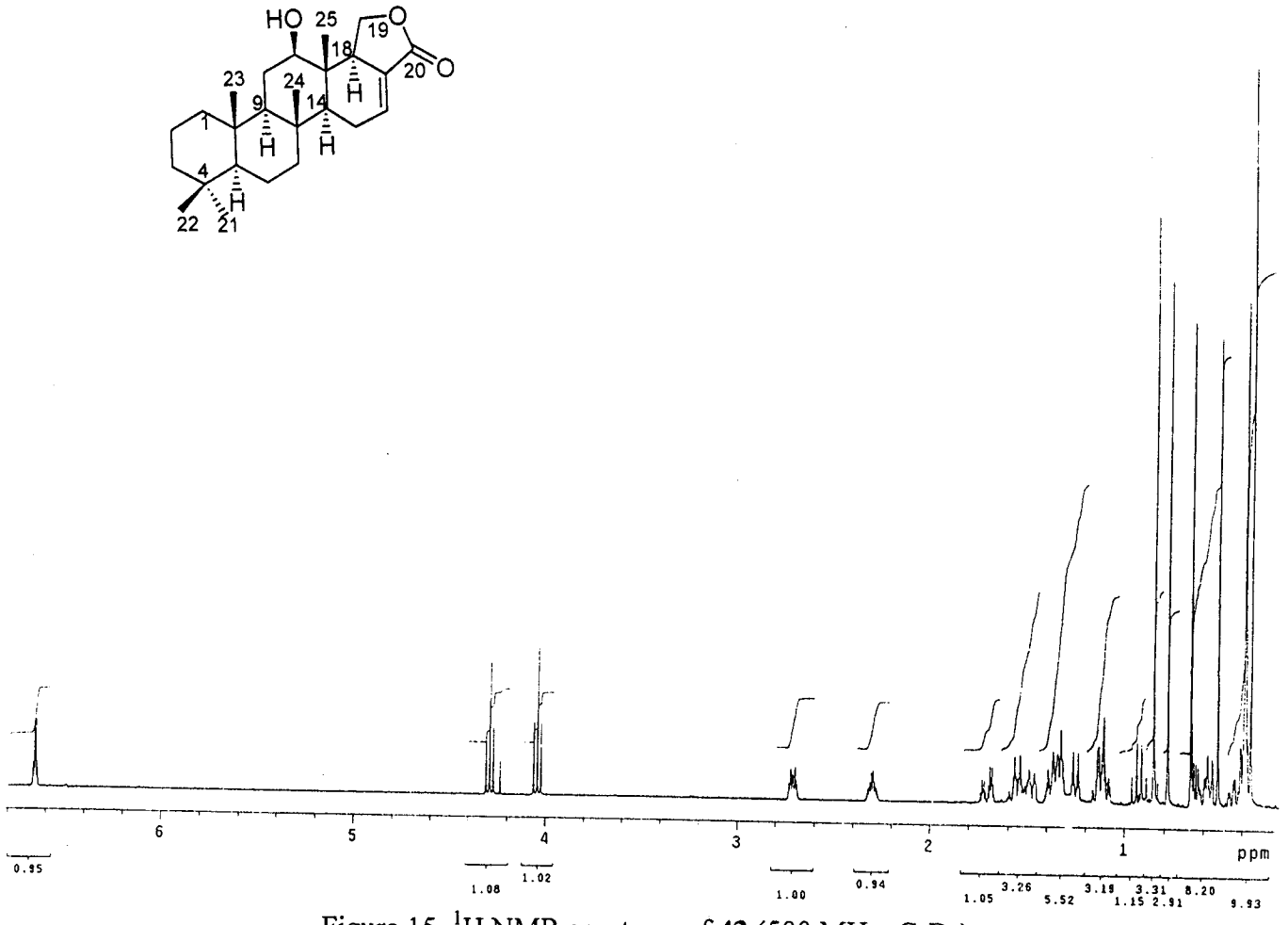


Figure 15 ^{1}H NMR spectrum of **43** (500 MHz; C_6D_6)

Table 6 NMR data (500 MHz for ^1H; in C_6D_6) of **42** and **43**

Position	δ_H (mult.; *J* in Hz)		δ_C (mult.)	
	42	**43**	**42**	**43**
1	Hax, 0.68 (m) Heq, 1.45 (m)	Hax, 0.60 (m) He, 1.48 (m)	39.7 (t)	40.1 (t)
2	Hax, 1.28 (m) Heq, 1.48 (m)	Hax, 1.39 (m) Heq, 1.56 (m)	18.1 (t)	18.9 (t)
3	Hax, 1.07 (m) Heq, 1.30 (m)	Hax, 1.11 (m) Heq, 1.36 (m)	42.1 (t)	42.3 (t)
4	-	-	33.4 (s)	33.4 (s)
5	0.58 (m)	0.57 (dd, 2.3, 12.4)	56.2 (d)	56.4 (d)
6	Hax, 1.13 (m) Heq, 1.32 (m)	Hax, 1.13 (m) Heq, 1.33 (m)	18.6 (t)	18.2 (t)
7	Hax, 0.46 (m) Heq, 1.25 (m)	Hax, 0.45 (m) Heq, 1.26 (ddd, 3.2, 3.2, 12.8)	41.1 (t)	41.2 (t)
8	-	-	37.2 (s)	37.1 (s)
9	0.59 (m)	0.40 (m)	58.0 (d)	58.4 (d)
10	-	-	37.4 (s)	37.5 (s)
11	Hax, 1.13 (m) Heq, 1.73 (ddd, 1.8, 4.3, 12.1)	Hax, 0.94 (m) Heq, 1.14 (m)	23.3 (t)	27.5 (t)
12	4.46 (dd, 4.3, 11.5)	2.72 (ddd, 4.5, 5.0, 11.4)	82.4 (d)	80.6 (d)
13	-	-	38.7 (s)	39.8 (s)
14	0.74 (m)	0.66 (m)	53.2 (d)	53.1 (d)
15	Ha, 1.70 (m) Hb, 1.71 (m)	Ha, 1.51 (m) Hb, 1.71 (dddd, 3.6, 4.1, 5.5, 20.1)	23.4 (t)	23.5 (t)
16	6.64 (ddd, 3.4, 3.6, 3.7)	6.67 (ddd, 3.6, 3.6, 3.6)	134.4 (d)	134.0 (d)
17	-	-	127.0 (s)	127.6 (s)

Table 6 (cont.)

Position	δ_H (mult.; *J* in Hz)		δ_C (mult.)	
	42	**43**	**42**	**43**
18	2.33 (dddd, 3.6, 3.9, 9.1, 9.2)	2.33 (dddd, 3.6, 4.1, 9.6, 9.6)	49.6 (d)	50.7 (d)
19	Ha, 3.82 (dd, 9.2, 9.3) Hb, 3.92 (dd, 9.1, 9.2)	Ha, 4.05 (dd, 9.6, 9.6) Hb, 4.30 (dd, 9.6, 9.6)	67.2 (t)	68.5 (t)
20	-	-	169.3 (s)	169.2 (s)
21	0.76 (s, 3H)	0.79 (s, 3H)	21.4 (q)	21.4 (q)
22	0.85 (s, 3H)	0.87 (s, 3H)	33.3 (q)	33.3 (q)
23	0.64 (s, 3H)	0.68 (s, 3H)	16.5 (q)	16.7 (q)
24	0.56 (s, 3H)	0.54 (s, 3H)	16.6 (q)	16.5 (q)
25	0.48 (s, 3H)	0.38 (s, 3H)	8.7 (q)	7.6 (q)
26	-	-	169.3 (s)	-
27	1.54 (s, 3H)	-	20.8 (q)	-

3.2.2 The manoalide-sesterterpenes

3.2.2.1 The structure elucidation of 44

Compound **44** was obtained as viscous colorless liquid (4 mg) from the hexane-soluble material of the second-expedition specimen by consecutive chromatographic techniques using SiO_2 column (EtOAc:acetone:hexane = 20:5:75), semi-preparative normal phase HPLC (SiO_2, isocratic 5% isopropanol in hexane; UV detector, 220 nm) and reverse phase HPLC (ODS, isocratic 75% aqueous CH_3CN; UV detector, 220 nm).

The ESI mass spectrum of **44** (Figure 57) shows a molecular peak at *m/z* 509 ($[M+Na]^+$). Thus, along with 29 carbon signals observed from its ^{13}C NMR spectrum, the molecular formula was suggested as $C_{29}H_{42}O_6$. The proposed molecular formula was comfirmed by the $[M+Na]^+$ peak at *m/z* 509.2898 in the HR-ESI mass spectrum (calc for $C_{29}H_{42}O_6Na$ 509.2868). The proposed molecular formula requires the unsaturation degree of nine. The ^{13}C NMR spectrum (Figure 16) indicates the presence of three carbonyl carbons and eight sp^2 carbons, leaving two sites of unsaturation unassigned. Therefore, two ring systems are required for **44**. The IR spectrum (Figure 58) shows the major absorption bands at ν 1755 and 1225 cm^{-1}, suggesting the presence of lactone functionality. The UV spectrum (Figure 59) shows the maximal absorption at λ 224 nm (log ε 3.72).

The ^1H NMR spectrum of **44** (Figure 17) shows the signals of three olefinic protons, one oxygenated methine proton, two oxygenated methylene groups and six methyl groups; thus, three spin systems were deduced. The first spin system is an α,β-unsaturated γ-lactone moiety with an acetoxy methylene substituent. This ring is composed of the signals at δ 5.62 (ddd, J = 1.6, 1.6, 3.4 Hz, H-2), coupling to the signal at δ 4.26 (m, H-4). The quarternary carbons of the lactone ring, i.e., the carbonyl carbon and the β carbon, were found resonating at δ 170.6 (C-1) and 164.6 (C-3), respectively, as determined by the analysis of the HMBC spectrum (see Table 8). The acetoxy methylene group is composed of the methylene protons at δ 4.15 (dd, J = 1.6, 16.2 Hz, H-25a) and 4.25 (dd, 1.6, 16.2 Hz, H-25b), one carbonyl carbon at δ 169.1 (C-28) and one methyl group at δ 1.53 (s, 3H, H-29).

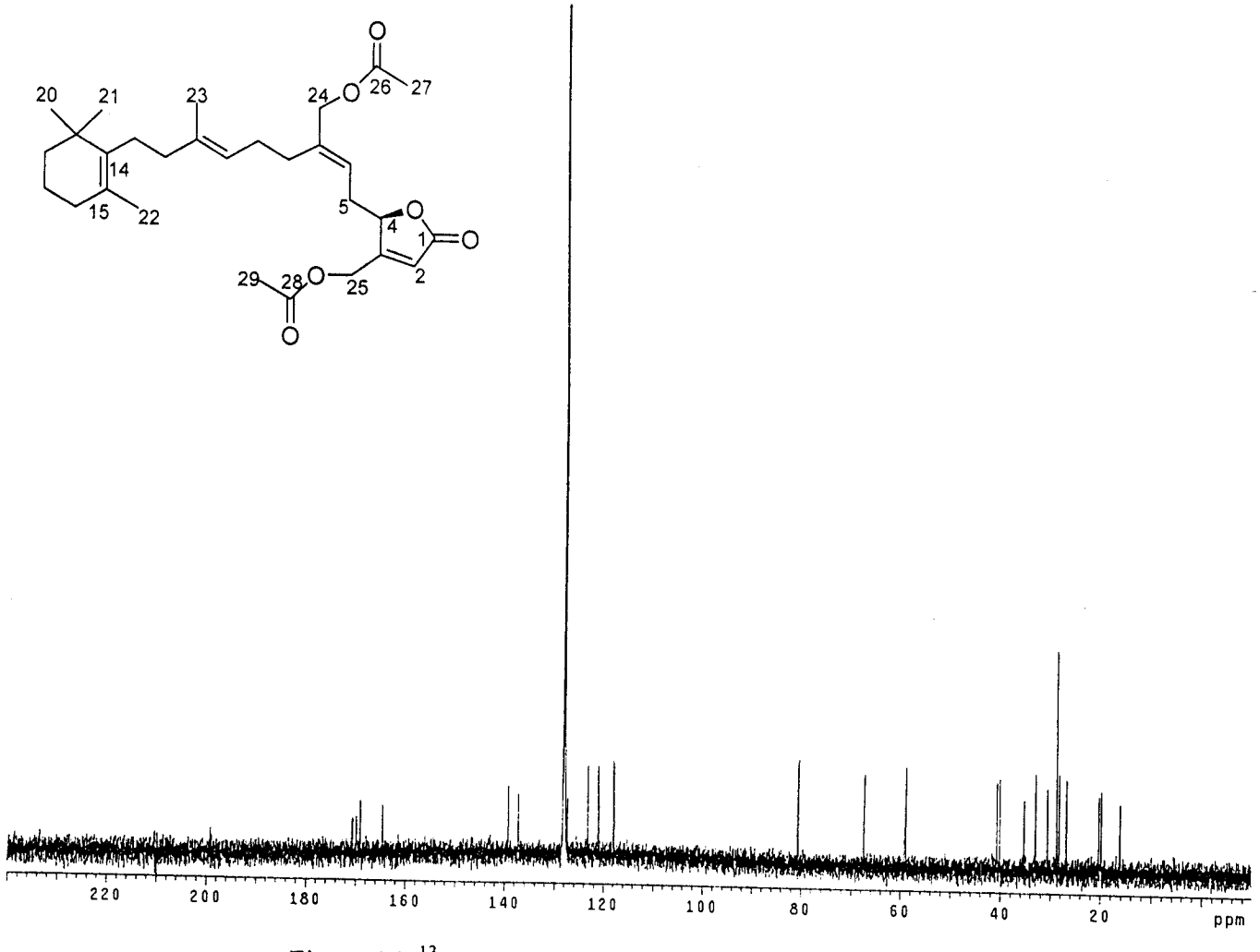


Figure 16 ^{13}C NMR spectrum of **44** (125 MHz; C_6D_6)

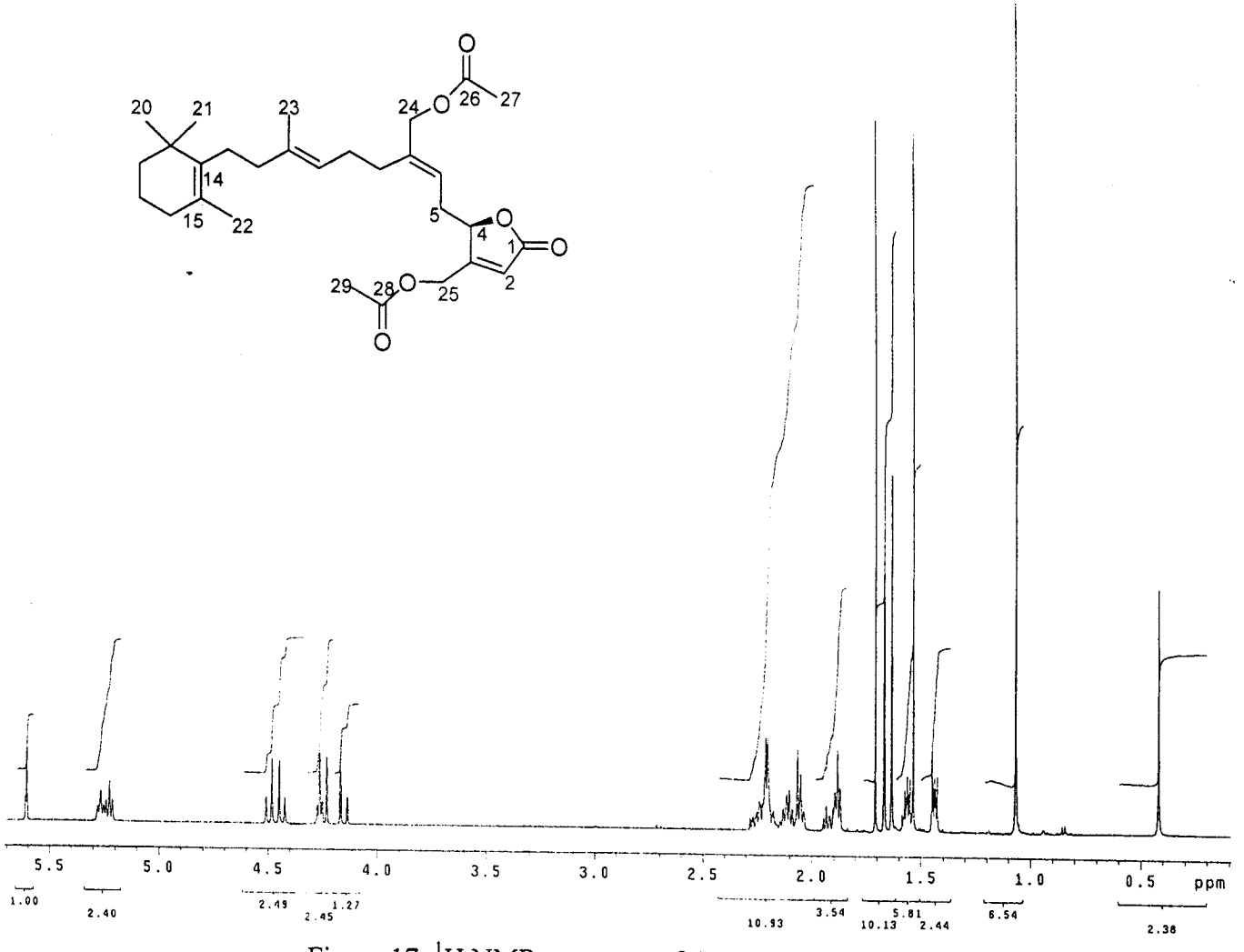
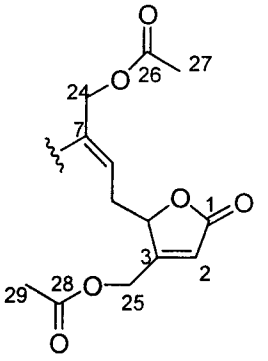


Figure 17 ^{1}H NMR spectrum of **44** (500 MHz; C_6D_6)

The HMBC correlations from C-2 (δ 117.2) and C-3 (δ 164.6) to H-25a and H-25b helped the connection of this acetoxy methylene onto C-3 (Table 8).

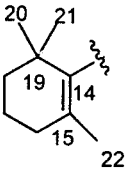
In addition, the γ-carbon of the α,β-unsaturated γ-lactone moiety also linked to one methylene, which was then extended to an olefin and the other acetoxy methylene. By means of the ^{1}H-^{1}H COSY spectral analysis, the extension from H-4 through the methylene (δ 1.93, ddd, J = 6.5, 7.3, 15.3 Hz, H-5a and δ 2.26, ddd, J = 5.0, 7.3, 15.3 Hz, H-5b) to the olefin (δ 5.23, t, J = 7.3 Hz, H-6) was achieved. The chemical shifts of the olefin carbons were assigned to be 121.1 (C-6) and 139.2 (C-7) ppm, on the basis of HMBC analysis. Also by the HMBC analysis, the acetoxy methylene moiety (δ 4.43, d, J = 12.4 Hz, H-24a; δ 4.49, d, J = 12.4 Hz, H-24b; δ 169.8, C-26; δ1.71, s, H-27) were placed onto C-7, thus furnished fragment **A** as shown.



A

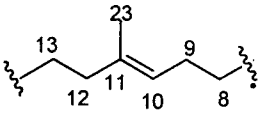
The second spin system is a tetrasubstituted cyclohexenyl moiety. Six methylene protons at δ 1.88 (dd, 2H, J = 6.2, 6.4 Hz, H-16), 1.56 (m, 2H, H-17) and 1.44 (m, 2H, H-18), were connected by means of ^{1}H-^{1}H COSY analysis.

These were further linked to an sp^3 carbon at δ 35.2 (C-19) and two olefinic carbons at δ137.2 (C-14) and δ 127.3 (C-15). The crucial HMBC correlation within this cyclohexeneyl moiety include those from C-19 to all the three methylene groups, from C-14 to H-16 and from C-15 to H-16. The placements of three methyl groups on C-19 and C-15 were assigned according to the C-H correlations observed in the HMBC spectrum. The two methyl groups resonating at δ 1.07 (s, 6H, H-20, H-21) correlate with C-19, whereas another at δ 1.63 (s, 3H, H-22) does with C-15. This structural part is shown as fragment **B**.



B

The last spin system is a hexenyl bridge, composed of the olefinic signals at δ 123.2 (C-10) and 137.2 (C-11). This olefin is linked to two ethylenes and one methyl groups. The first ethylene moiety (δ 2.06, m, H-8a; 2.17, m, H-8b; 2.23, m, 2H, H-9) is placed on C-10, whereas the second one (δ 2.19, m, 2H, H-12; 2.05, m, 2H, H-13) is on C-11, as determined from the HMBC spectrum. Due to the C-H long-range correlation from C-9 (δ 28.3), C-10 (δ 123.2) and C-11 (δ 137.2) to the methyl group at δ 1.67 (s, 3H, H-23), this methyl group (H-23) is placed on C-11. This spin system is shown as fragment **C**.



C

All the three fragments are linked on the basis of the analysis of C-H long-range correlations. The fragment **C** is used as a bridge, linking fragments **A** and **B**, due to the observation of the long-range correlations from C-14 and C-15 to H-12; from C-19 to H-13; from C-7 to H-8a and H-8b; from C-8 to H-6; and from C-8 to H-24a and H-24b (Figure 62). Therefore, the structure of **44** was proposed as shown below. It was designated as neomanoalide diacetate. The NMR spectral data are summarized in Tables 7 and 8.

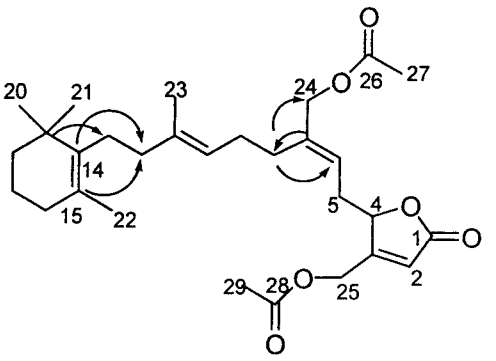


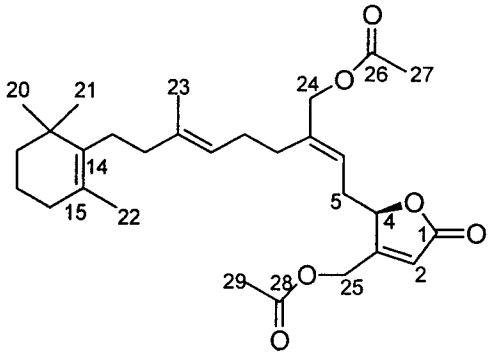
Figure 18 Crucial HMBC correlations from ^{13}C to ^{1}H of **44**

The stereochemistry of **44** was determined by means of a close observation of chemical shifts and CD spectral analysis. The up-field shift of C-8 (δ 28.8) and down-field shift of C-24 (δ 67.4), as compared to those reported by de Silva and Scheuer (1981), suggest the electronic repulsion on C-8, thus indicating the *E*-

configuration of the $\Delta^{6,7}$ olefin. Furthermore, the dipolar couplings observed among H-24a, H-24b and H-6 support the proposed configuration.

Similarly, the configuration of $\Delta^{10,11}$ double bond is also *E*, inferred from the up-field signal at 16.2 ppm of C-23. Such methyl group would resonate at lower field in *Z*-isomer. For example, the chemical shift of the methyl signal in (*E*)-3 methyl-3-hexene is at 15.7 ppm, whereas that of *Z* one is at 22.9 ppm (Yunker and Scheuer, 1978).

The absolute configuration at C-4 of **44** was determined by means of the CD spectral analysis. The CD spectrum of **44** (Figure 19) shows the first negative Cotton effect with $[\theta]_{218}$ of –5504, indicating that the configuration at C-4 is *R*, according to the octant rule (Eliel and Wilen, 1994). Furthermore, the negative specific rotation of **44** ($[\alpha]_D^{25}$ –33°) corresponds well with those previously reported for the neomanoalides (Kobayashi *et al.*, 1994), thus confirming the proposed configuration.



44

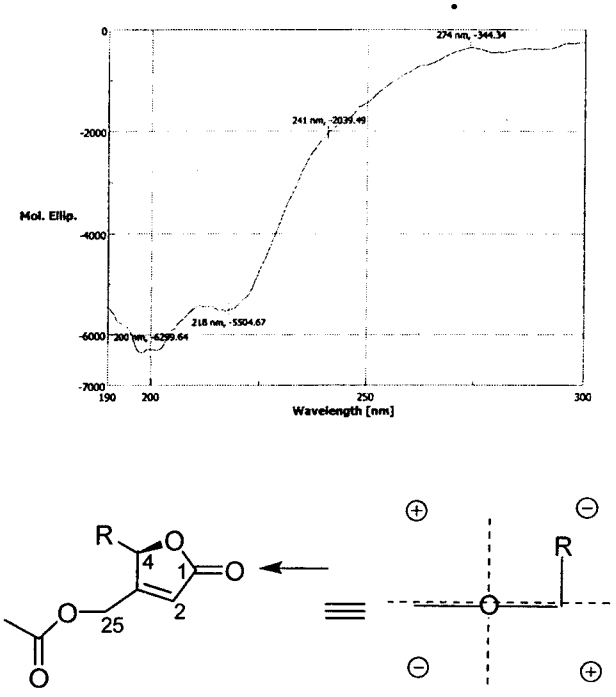


Figure 19 CD spectrum of compound **44**

Compound **44** is identified as (*E*)-neomanoalide diacetate. This compound was originally derived as part of the structure elucidation of (*E*)-neomanoalide (de Silva and Scheuer, 1981). However, all the spectral data, physical properties, and biological activities have never been reported. Therefore, here is the first report of naturally occuring 4*R*-(*E*)-neomanoalide diacetate.

3.2.2.2 The structure elucidation of 45

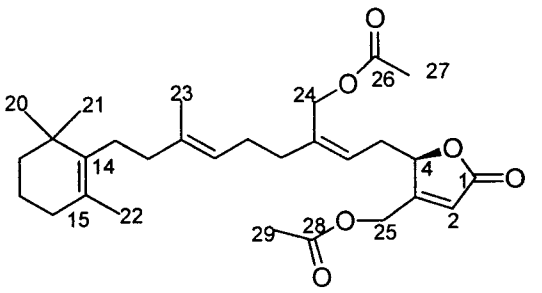
Also viscous colorless liquid similar to **44**, compound **45** (7 mg) was obtained along with **44** by successive chromatographic techniques using SiO_2 column (EtOAc:acetone:hexane = 20:5:75), semi-preparative normal phase HPLC (SiO_2, isocratic 5% isopropanol in hexane; UV detector, 220 nm) and reverse phase HPLC (ODS, isocratic 75% aqueous CH_3CN; UV detector, 220 nm), respectively.

The molecular formula of **45** was proposed to be $C_{29}H_{42}O_6$ as deduced from its molecular peak at *m/z* 509 ($[M+Na]^+$) in the ESI mass spectrum (Figure 65), along with 29 carbon signals observed from its ^{13}C NMR spectrum. The proposed molecular formula was comfirmed by the $[M+Na]^+$ peak at *m/z* 509.2794 in the HR-ESI mass spectrum (calc 509.2868). Similar to **44**, the nine-degree of unsaturation are assigned as three carbonyl carbons, four carbon-carbon double bonds and two ring systems. The lactone functionality was also observed as the major absorption bands at ν 1755 and 1225 cm^{-1} in its IR spectrum (Figure 66). The UV spectrum (Figure 67) shows the absorption maximum at λ 224 (log ε 3.72).

Except some slight chemical shift differences, the ^{13}C and ^{1}H NMR spectra (Figures 20 and 21, respectively) of **45** were almost identical to those of **44**. Here, the two methylene protons previously assigned to H-24a and H-24b in **44**, were found to collide into a singlet resonating at δ 4.52. Also the signal at δ 35.6 that

was assigned to C-8 of **45** was found to shift toward lower field than that of **44**, whereas the signal at δ 61.4, assigned to C-24, otherwise moved toward higher field. These evidences suggest that **44** and **45** do in fact share the similar empirical structure, but differ only at the $\Delta^{6,7}$ configuration, which was assigned as *Z* for **45**. This assignment is supported by the comparison with that previously reported by de Silva and Scheuer (1981).

Also similar to **44**, the first negative Cotton effect ($[\theta]_{214.5}$ −10485), in its CD spectrum (Figure 71) was observed, thus indicating an *R* configuration at C-4. Compound **45** thus was proposed as 4*R*-(*Z*)-neomanoalide diacetate as shown below. The compound is first reported here as a new naturally-occurring sesterterpene; however, also similar to **44,** it was reported non-informatively by de Silva and Scheuer (1981). The NMR spectral data of **45** are summarized in Tables 7 and 8.



45

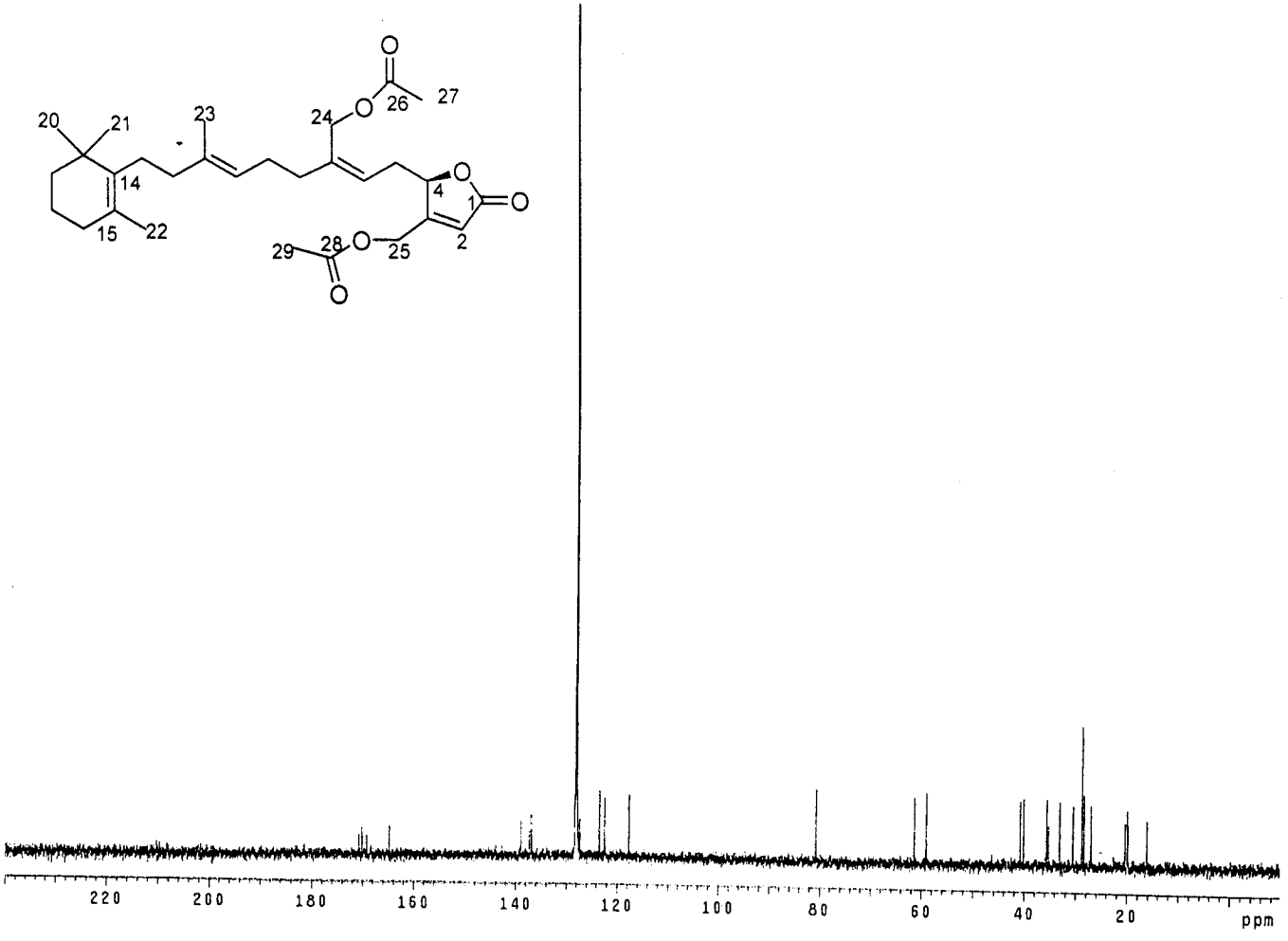


Figure 20 ^{13}C NMR spectrum of **45** (125 MHz; C_6D_6)

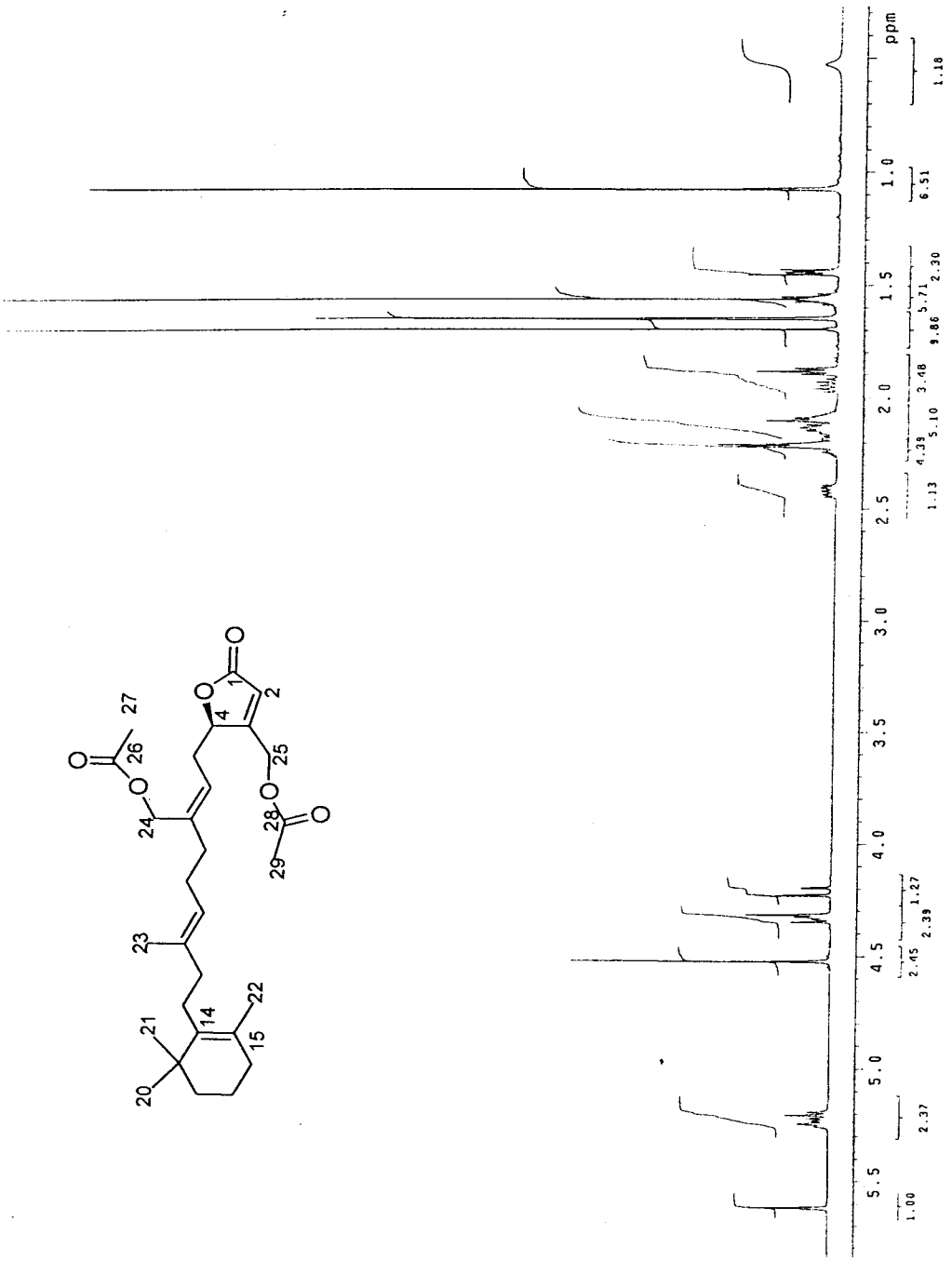


Figure 21 ^{1}H NMR spectrum of **45** (500 MHz; C_6D_6)

3.2.2.3 The structure elucidation of 46

Compound **46** was re-crystallized as a white needle crystal (7 mg) from the mixture of isopropanol:hexane (1:5). It was isolated from hexane-soluble material of the second-expedition specimen by successive chromatographic techniques using SiO_2 column (EtOAc:acetone:hexane = 20:5:75) followed by semi-preparative normal phase HPLC (SiO_2, isocratic 2% isopropanol in hexane; UV detector, 220 nm), respectively.

The ESI mass spectrum of **46** (Figure 72) shows a molecular peak at *m/z* 481 ($[M+Na]^+$). Together with the numbers of carbons and protons observed from the ^{13}C and ^{1}H spectra (Figures 22 and 23), a molecular formula of $C_{27}H_{38}O_6$ was suggested. Six of the nine-degree of unsaturation calculated from the molecular formula of **46** are taken up in four carbon-carbon double bonds and two carbonyl carbons; thus this molecule is composed of three ring systems. The IR spectrum (Figure 73) exhibits the absorption bands at ν 3430 cm^{-1} for a hydroxyl group and at ν 1790, 1770 and 1235 cm^{-1} for ester functionalities. The UV spectrum (Figure 74) shows the absorption maximum at λ 224 nm (log ε 3.81).

Whereas both ^{13}C and ^{1}H NMR spectra of **46** (Figures 22 and 23) are not entirely identical to those of **44** and **45**, the western part of the structure, i.e. the hexenyl cyclohexene part (C-8 - C-23), was able to be observed. Thus, the three compounds evidently share similar structural unit, suggesting the possibility that all the three belong to the same chemical family. The eminent differences

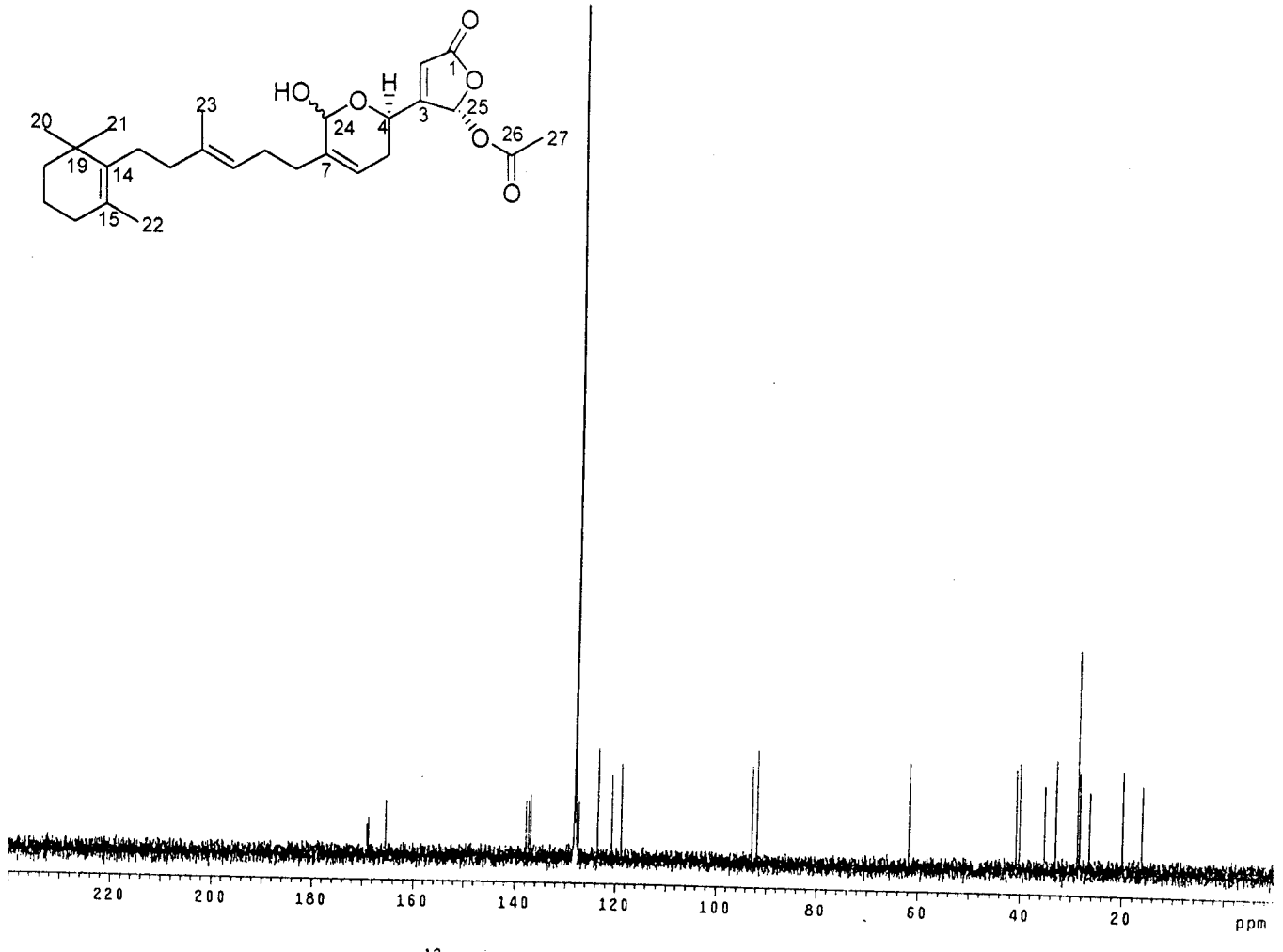


Figure 22 ^{13}C NMR spectrum of **46** (125 MHz; C_6D_6)

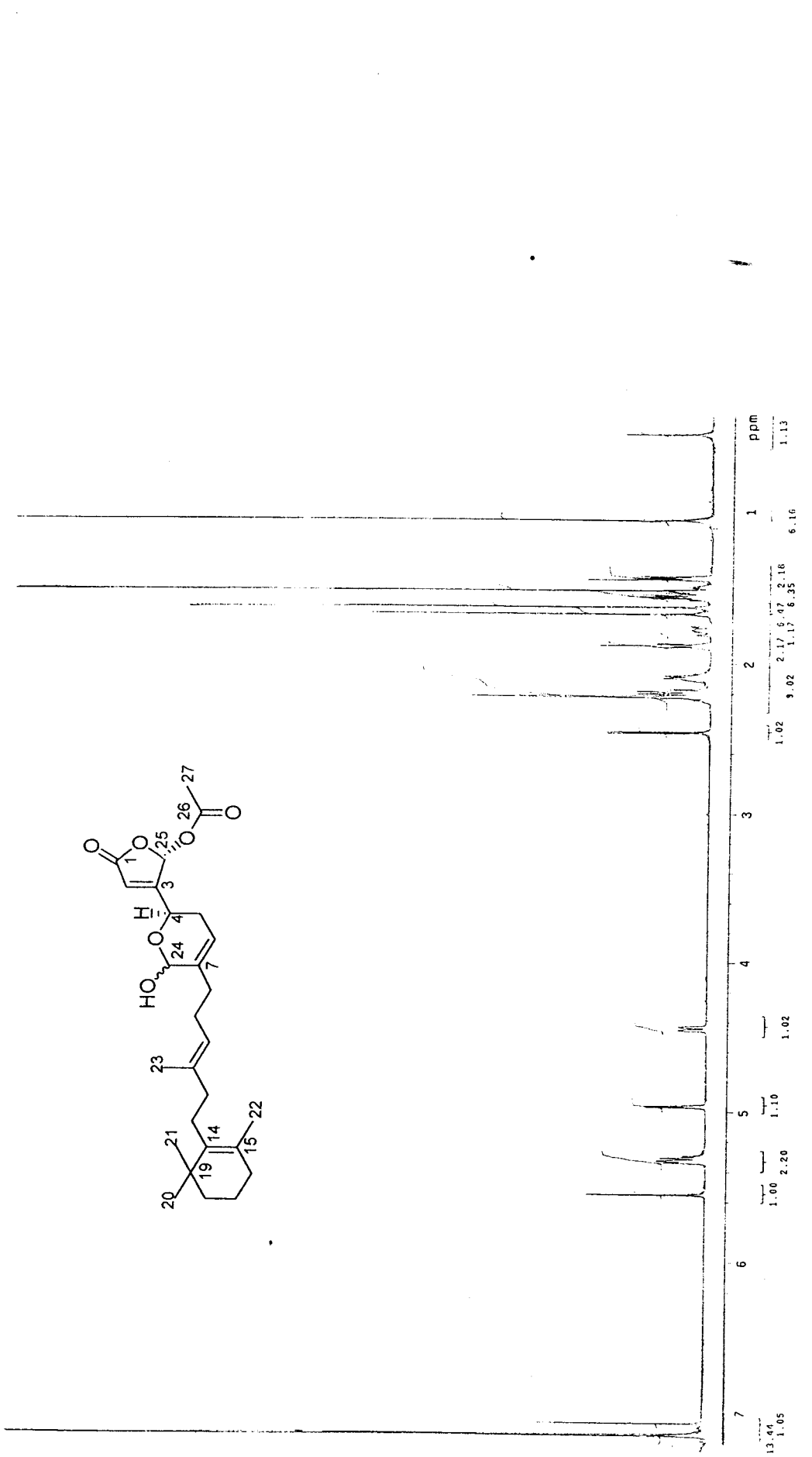


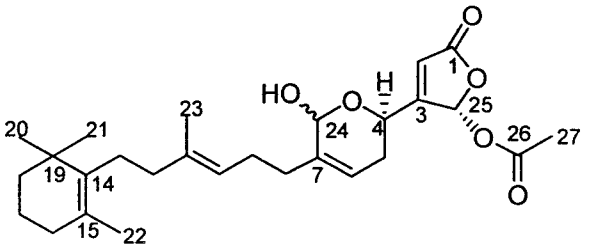
Figure 23 ^{1}H NMR spectrum of **46** (500 MHz; C_6D_6)

observed in the ^{1}H NMR spectrum of **46** include the signal of two olefinic protons, two acetals, one carbonol proton, two methylene protons and one acetate methyl. Connecting all of these signals by means of HMBC spectral analysis, led to the establishment of two structural moieties.

The first moiety was proposed as an α,β-unsaturated γ-lactone ring, which is composed of the signals at δ 5.55 (dd, J = 1.0, 2.0 Hz, H-2) coupled to the signal at δ 7.07 (br.s, H-25), and one carbonyl and one β carbons resonating at δ 169.1 (C-1) and 165.4 (C-3), respectively, as determined by the analysis of HMBC spectrum (Figure 77). The chemical shifts of 7.07 ppm (H-25) and 92.8 ppm (C-25), characteristic to the acetal functionality, suggest that this position was linked to the acetoxy group (δ 169.1, C-26 and 19.9, C-27 in ^{13}C NMR; and δ 1.53, s, 3H, H-27 in ^{1}H NMR). The HMBC correlation from C-26 to H-25 confirms this connectivity.

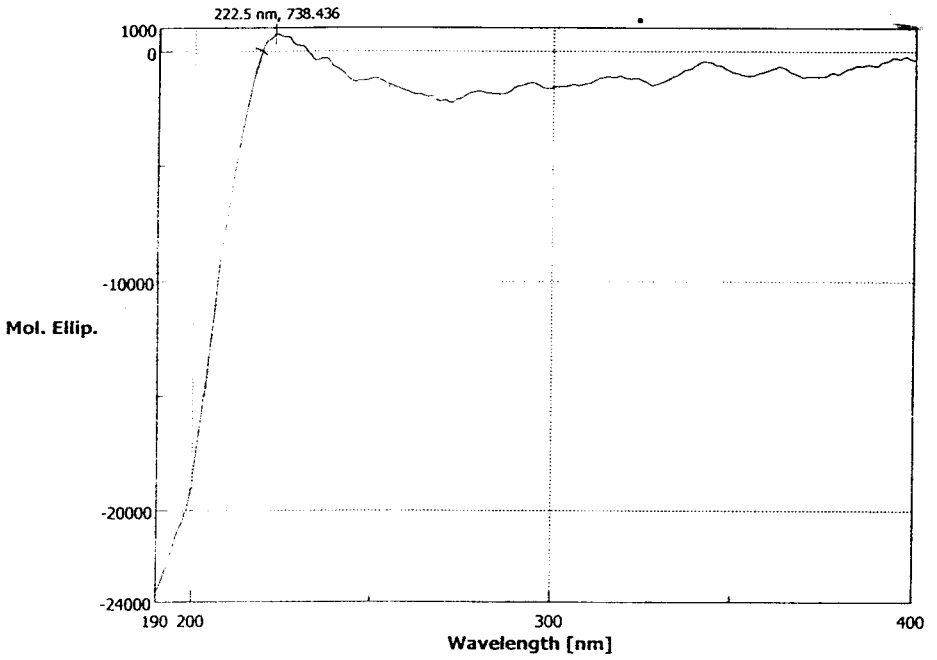
The other moiety is a δ-hydroxy lactone ring, composed of the signal at δ 4.46 (ddd, J = 2.0, 3.2, 11.4 Hz, H-4), which then further couples to the signal at δ 1.51 (m, H-5a) and 1.78 (br.ddd, J = 3.4, 11.4, 17.0 Hz, H-5b), then to one olefinic proton at δ 5.33 (m, H-6), and finally to the other acetal proton at δ 4.98 (d, J = 4.8 Hz, H-24). Additionally, the ^{1}H-^{1}H COSY spectrum (Figure 75) also shows a transverse coupling between the signal of H-24 and an exchangeable proton at δ 2.45 (d, J = 4.8 Hz, 24-OH). The coupling constant of 11.4 Hz of H-4 indicates the orientation of H-4 as axial. The allylic coupling between H-4 and H-2 suggests the

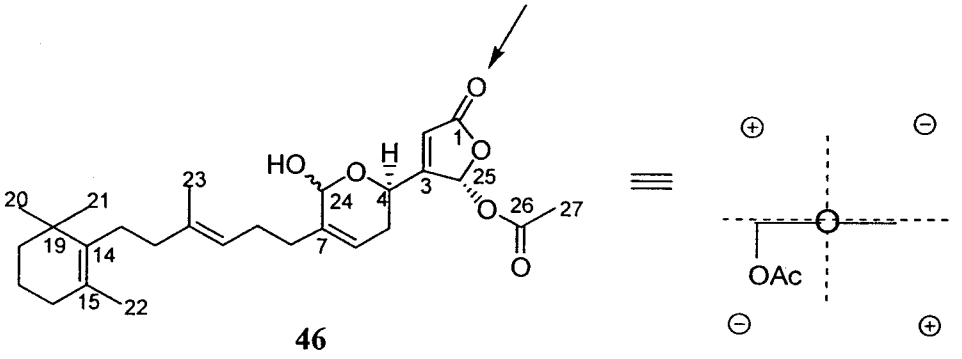
connection between the two moieties as shown. Finally the hexenyl cyclohexene portion of C-8-C-25 was linked to C-7 and structure of **46** was established as shown. The NMR spectral data are summarized in Table 7.



46

Compound **46** was identified as a known compound, manoalide-25-acetate. The proton and carbon assignments of **46** were confirmed by comparison with the data reported by Cambie and Craw (1988). The absolute configuration at C-25 of **46** was determined by the CD exciton chirality method. As the CD spectrum of **46** (Figure 24) reveals the first negative Cotton effect ($[\theta]_{200}$ –20000), indicating the *S* configuration at C-25. On the other hand, the absolute configuration on the pyranol ring is unable to be determined directly. Here, the configuration on C-4 was proposed as *R* by comparison of the positive specific rotation ($[\alpha]_D^{25}$ +25°) along with the biosynthetic consideration of natural manoalide family (Amoo, Bernardo and Weigele, 1988; Butler and Capon, 1992; Kobayashi *et al.*, 1994). We proposed the structure of **46** with the first report on the absolute configuration at C-25 as 4*R*,25*S*-manoalide-25-acetate.





46

Figure 24 The CD spectrum of **46**

Table 7 NMR data (500 MHz for ^1H; in C_6D_6) of **44**, **45** and **46**

position	δ_H (mult.; *J* in Hz)			δ_C (mult.)		
	44	**45**	**46**	**44**	**45**	**46**
1	-	-	-	170.6 (s)	170.7 (s)	169.1 (s)
2	5.62 (ddd, 1.6, 1.6, 3.4)	5.62 (ddd, 1.6, 1.8, 3.4)	5.55 (dd, 1.0, 2.0)	117.2 (d)	117.7 (d)	118.8 (d)
3	-	-	-	164.6 (s)	164.9 (s)	165.4 (s)
4	4.26 (m)	4.33 (m)	4.46 (ddd, 2.0, 3.2, 11.4)	80.5 (d)	80.7 (d)	61.8 (d)
5	Ha, 1.93 (ddd, 6.5, 7.3, 15.3)	Ha, 1.94 (ddd, 6.9, 7.3, 15.1)	Ha, 1.51 (m)	30.7 (t)	30.5 (t)	28.3 (t)
	Hb, 2.26 (ddd, 5.0, 7.3, 15.3)	Hb, 2.41 (ddd, 4.4, 7.3, 15.1)	Hb, 1.78 (br.ddd, 3.2, 11.4, 17.0)			
6	5.23 (t, 7.3)	5.21 (t, 7.3)	5.33 (m)	121.1 (d)	122.4 (d)	120.7 (d)
7	-	-	-	139.2 (s)	138.9 (s)	137.8 (s)
8	Ha, 2.06 (m)	Ha, 2.09 (m)	2.08 (m)	28.8 (t)	35.6 (t)	33.1 (t)
	Hb, 2.17 (m)	Hb, 2.13 (m)				
9	2.23 (m, 2H)	2.19 (m, 2H)	2.18 (m, 2H)	28.3 (t)	28.4 (t)	28.4 (t)
10	5.26 (dd, 6.2, 6.9)	5.24 (ddd, 1.1, 6.2. 7.1)	5.30 (ddd, 0.9, 6.2, 6.9)	123.2 (d)	123.4 (d)	123.5 (d)
11	-	-	-	137.2 (s)	136.8 (s)	136.9 (s)

Table 7 (cont.)

position	δ_H (mult.; *J* in Hz)			δ_C (mult.)		
	44	**45**	**46**	**44**	**45**	**46**
12	2.19 (m, 2H)	2.20 (m, 2H)	2.20 (m, 2H)	40.8 (t)	40.8 (t)	40.8 (t)
13	2.05 (m, 2H)	2.10 (m, 2H)	2.21 (m, 2H)	26.9 (t)	27.0 (t)	26.4 (t)
14	-	-	-	137.2 (s)	137.3 (s)	137.3 (s)
15	-	-	-	127.3 (s)	127.3 (s)	127.3 (s)
16	1.88 (dd, 6.2, 6.4, 2H)	1.88 (dd, 6.2, 6.4, 2H)	1.88 (dd, 6.2, 6.4, 2H)	33.0 (t)	33.0 (t)	33.0 (t)
17	1.56 (m, 2H)	1.57 (m, 2H)	1.56 (m, 2H)	19.9 (t)	20.0 (t)	19.9 (t)
18	1.44 (m, 2H)	1.44 (m, 2H)	1.44 (m, 2H)	40.2 (t)	40.2 (t)	40.1 (t)
19	-	-	-	35.2 (s)	35.2 (s)	35.2 (s)
20	1.07 (s, 3H)	1.07 (s, 3H)	1.06 (s, 3H)	28.8 (q)	28.8 (q)	28.8 (q)
21	1.07 (s, 3H)	1.07 (s, 3H)	1.06 (s, 3H)	28.8 (q)	28.8 (q)	28.8 (q)
22	1.63 (s, 3H)	1.65 (s, 3H)	1.63 (s, 3H)	20.0 (q)	20.0 (q)	20.0 (q)
23	1.67 (s, 3H)	1.65 (s, 3H)	1.68 (s, 3H)	16.2 (q)	16.2 (q)	16.2 (q)
24	Ha, 4.43 (d, 12.4)	4.52 (s)	4.98 (d, 4.8)	67.4 (t)	61.4 (t)	91.8 (d)
	Hb, 4.49 (d, 12.4)					

Table 7 (cont.)

position	δ_H (mult.; *J* in Hz)			δ_C (mult.)		
	44	**45**	**46**	**44**	**45**	**46**
25	Ha, 4.15 (dd, 1.6, 16.2) Hb, 4.25 (dd, 1.6, 16.2)	Ha, 4.21 (ddd, 0.6, 1.8, 16.2) Hb, 4.33 (dd, 1.6, 16.2)	7.07 (br.s)	58.9 (t)	59.0 (t)	92.8 (d)
26	-	-	-	169.8 (s)	170.1 (s)	168.7 (s)
27	1.71 (s, 3H)	1.69 (s, 3H)	1.52 (s, 3H)	20.4 (q)	20.4 (q)	19.9 (q)
28	-	-	-	169.1 (s)	169.1 (s)	-
29	1.53 (s, 3H)	1.56 (s, 3H)	-	19.8 (q)	19.8 (q)	-
24-OH	-	-	2.45 (d, 4.8)	-	-	-

Table 8 HMBC correlations ($^{13}C \longrightarrow {}^{1}H$) of **44** and **45**

Position	HMBC correlation ($^{13}C \longrightarrow {}^{1}H$)	
	44	**45**
1	H-2, H-4	H-2, H-4, H-25a, H-25b
2	H-25a, H-25b	H-25a, H-25b
3	H-2, H-5a, H-5b, H-6, H-25a, H-25b	H-2, H-5a, H-5b, H-25a, H-25b
4	H-2, H-5a, H-5b, H-25a, H-25b	H-2, H-5a, H-5b,H-6, H-25a, H-25b
5	H-4, H-6	H-4, H-6
6	H-4, H-5a, H-5b, H-8a, H-8b, H-9, H-24a, H-24b	H-4, H-5a, H-5b, H-24
7	H-5a, H-5b, H-8a, H-8b, H-9, H-24a, H-24b	H-5a, H-5b, H-8a, H-8b, H-24
8	H-6, H-24a, H-24b	H-6, H-24
9	-	H-23
10	H-8a, H-8b, H-12, H-13, H-23	H-8a, H-8b, H-9, H-13, H-23
11	-	H-9, H-13, H-23
12	H-23	-
13	H-10	H-10
14	H-12, H-13, H-16, H-20, H-21	H-12, H-16, H-18, H-20, H-21, H-22
15	H-16, H-17, H-22	H-12, H-16, H-17, H-20, H-21, H-22
16	H-17, H-18, H-22	H-17, H-18
17	-	-
18	H-16, H-20, H-21	H-16, H-20, H-21
19	H-16, H-17, H-18, H-20, H-21	H-13, H-16, H-17, H-18, H-20, H-21, H-22
20	H-18, H-21	H-18, H-21
21	H-18, H-20	H-18, H-20
22	-	H-16

Table 8 (cont.)

Position	HMBC correlation ($^{13}C \longrightarrow ^{1}H$)	
	44	**45**
23	H-10	H-10
24	H-6	H-6
25	-	-
26	H-24a, H-24b, H-27	H-24, H-27
27	-	-
28	H-25a, H-25b, H-29	H-25a, H-25b, H-29
29	-	-

3.3 Biological activities of the isolated compounds

All the eight sesterterpenes isolated from the sponge *Brachiaster* sp. were assessed for antituberculosis activity against *Mycobacterium tuberculosis* ($H_{37}Ra$) using the Microplate alamar blue assay, and for cytotoxic activity against four cell lines, including MCF-7, HeLa, HT-29 and KB, using SRB assay. The results are presented in Table 9. The MICs and IC_{50} values were obtained as a microgram per milliliter unit, and later converted to a micromolar unit, which is preferable for potency comparison.

The results demonstrate that all compounds exhibit antituberculosis activity against *M. tuberculosis* with MICs ranging from 3 to 117 μM (1.56-50 μg/mL). Compounds **40**, **18**, **41** and **46** show the most potent antituberculosis activity with MICs of 3-7 μM, whereas compounds **42**, **43**, **44** and **45** are mildly active. On the other hand, the significant cytotoxicity was observed only with compounds **18** and **46**, with IC_{50} in all targeted cell lines lower than 1 μg/mL. Among all the isolated compounds reported, **18** was the only agent previously tested elswhere for antituberculosis activity with MIC in a comparable potency of 6.25 μg/mL (El Sayed *et al.*, 2000). Here, the antituberculosis activity of the other seven compounds is first reported along with their cytotoxicity against cancer cell lines.

Evidently, among the five scalaranes, the furanoid ring E significantly affects the potency of antituberculosis activity. It was reported by Crews and Bescansa (1986) that the oxygen functionality in the vicinity of C-19 and C-20 of

scalaranes may be a structural feature required for biotoxicity. Particularly, the 19-acetal moiety seem to influence antituberculosis potency; i.e., the MICs of **40**, **18** and **41**, which bear the 19-acetal moiety, are ranging from 3 to 6 μM, whereas the other two scalaranes are much less active. Besides, the oxygenated pattern of C-12 possibly assert the complementarity of such activity to the certain extent. However, with only two pairs of such structure (**18** versus **41** and **42** versus **43**), an absolute conclusion is yet unable to be drawn.

In the manoalide-family, compound **46** shows the most potent antituberculsis activity (MIC 7 μM), whereas **44** and **45** show moderate activity (MICs 26 and 51 μM, respectively). It was observed that several natural products containing α,β-unsaturated γ-lactone moiety exhibit interesting chemotherapeutic activities, including antituberculosis and cytotoxic activities, due to the alkylation by conjugate additions with essential biological nucleophiles, i.e., proteins and nucleic acid (Cantrell *et al.*, 2001).

Whereas, most of the isolated sesterterpenes reported have showed a good correlations between cytotoxic and antituberculosis activities, i.e., those with potent cytotoxicity are as well antituberculosis active and vice versa, interestingly, 12-deacetoxy-scalarin acetate (**40**) exhibit the otherwise potency. The compound is strongly antituberculosis active (MIC 4 μM), but slightly cytotoxic against all targeted cell lines (IC_{50} higher than 12 μM). Such selectivity indicate the potential of scalarane-type sesterterpenes as antituberculosis agents, and probably other

chemotherapeutic agents, especially when the appropriate structural modification (s) are carried out.

Table 9 Antituberculosis and cytotoxic activities of compounds **40**, **18**, **41**, **42**, **43**, **44**, **45** and **46**

Compounds	Anti-TB[a] MIC; μg/mL	Cytotoxicity (IC_{50} ± SEM; μg/mL)			
		MCF-7	HT-29	HeLa	KB
12-Deacetoxy-scalarin acetate (**40**)	1.56 (4)[b]	> 5 (> 12)[b]	> 5 (> 12)[b]	> 5 (> 12)[b]	> 5 (> 12)[b]
Heteronemin (**18**)	1.56 (3)[b]	0.14±0.04 (0.29±0.08)[b]	0.1-0.25 (0.2-0.51)[b]	0.1-0.25 (0.2-0.51)[b]	0.1-0.25 (0.2-0.51)[b]
Heteronemin acetate (**41**)	3.125 (6)[b]	3.42±0.16 (6.45±0.30)[b]	> 5 (> 9)[b]	NT[c]	> 5 (> 9)[b]
12-Epi-19-deoxyscalarin (**42**)	50 (117)[b]	> 5 (> 12)[b]	> 5 (> 12)[b]	2.5-5 (5.8-12)[b]	1.29±0.68 (3.01±1.59)[b]
12-Deacetyl-12-epi-19-deoxyscalarin (**43**)	6.25 (16)[b]	> 5 (> 13)[b]	> 5 (> 13)[b]	> 5 (> 13)[b]	> 5 (> 13)[b]
E-Neomanoalide diacetate (**44**)	25 (51)[b]	> 5 (> 10)[b]	> 5 (> 10)[b]	> 5 (> 10)[b]	> 5 (> 10)[b]
Z-Neomanoalide diacetate (**45**)	12.5 (26)[b]	2.91±0.06 (5.99±0.12)[b]	> 5 (> 10)[b]	> 5 (> 10)[b]	> 5 (> 10)[b]
Manoalide-25-acetate (**46**)	3.125 (7)[b]	$0.12\pm0.42\times10^{-2}$ ($0.26\pm0.92\times10^{-2}$)[b]	0.35±0.06 (0.76±0.13)[b]	0.77±0.14 (1.68±0.39)[b]	0.29±0.05 (0.63±0.11)[b]

[a] Isoniazid and kanamycin sulfate were used as standard drugs (MICs 0.3-0.7 and 3-9 μM, respectively)

[b] The numbers in parentheses denote the potency in μM (with SEM, if applicable)

[c] not tested

CHAPTER 4

CONCLUSION

The bioassay-guided fractionation of the Thai sponge *Brachiaster* sp., collected from Koh-Tao, Surat Thani, led to the isolation of three new naturally-occurring sesterterpenes, 12-deacetoxy-scalarin acetate (**40**), (*E*)-neomanoalide diacetate (**44**) and (*Z*)-neomanoalide diacetate (**45**), along with five known sesterterpenes, heteronemin (**18**), heteronemin acetate (**41**), 12-epi-19-deoxy-scalarin (**42**), 12-deacetyl-12-epi-19-deoxyscalarin (**43**) and manoalide-25-acetate (**46**).

The results from bioactivity evaluation showed that, among the eight isolated sesterterpenes, the most active antituberculosis agents are compounds **40**, **18**, **41** and **46** with MICs ranging from 3-7 μM, whereas the significant cytotoxicity was observed in compounds **18** and **46**. Furthermore, the correlation between the antituberculosis and cytotoxic activities is prominently evident. Most of the isolated compounds that show the potent antituberculosis activity are also strongly cytotoxic and vice versa. Thus, their antituberculosis activity could possibly, and simply, stem from the cytotoxicity. However, among these, 12-deacetoxy-scalarin acetate (**40**) shows the otherwise result. Its MIC of antituberculosis activity is at 4 μM whereas the IC_{50} of cytotoxicity is higher than 12 μM. The potent and selective activity of the compound indicates that the

scalarane-type sesterterpenes, in fact, can be well regarded as a group of potential lead compounds for the development of antituberculosis agents, at a time when the efficacy of certain currently available drugs is declining. In addition, the preliminary structure-activity relationship of the scalaranes suggested that the 19-acetal moiety influences the potency of antituberculosis activity to certain extent.

Overall, this work has demonstrated that Thai marine organisms are among the potential sources of antituberculosis agents, as well as several agents of chemotherapeutic uses that may be useful in drug development. The further exploring will, therefore, yield compounds with greater efficacy and specificity for the treatment of many human diseases.

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APPENDIX

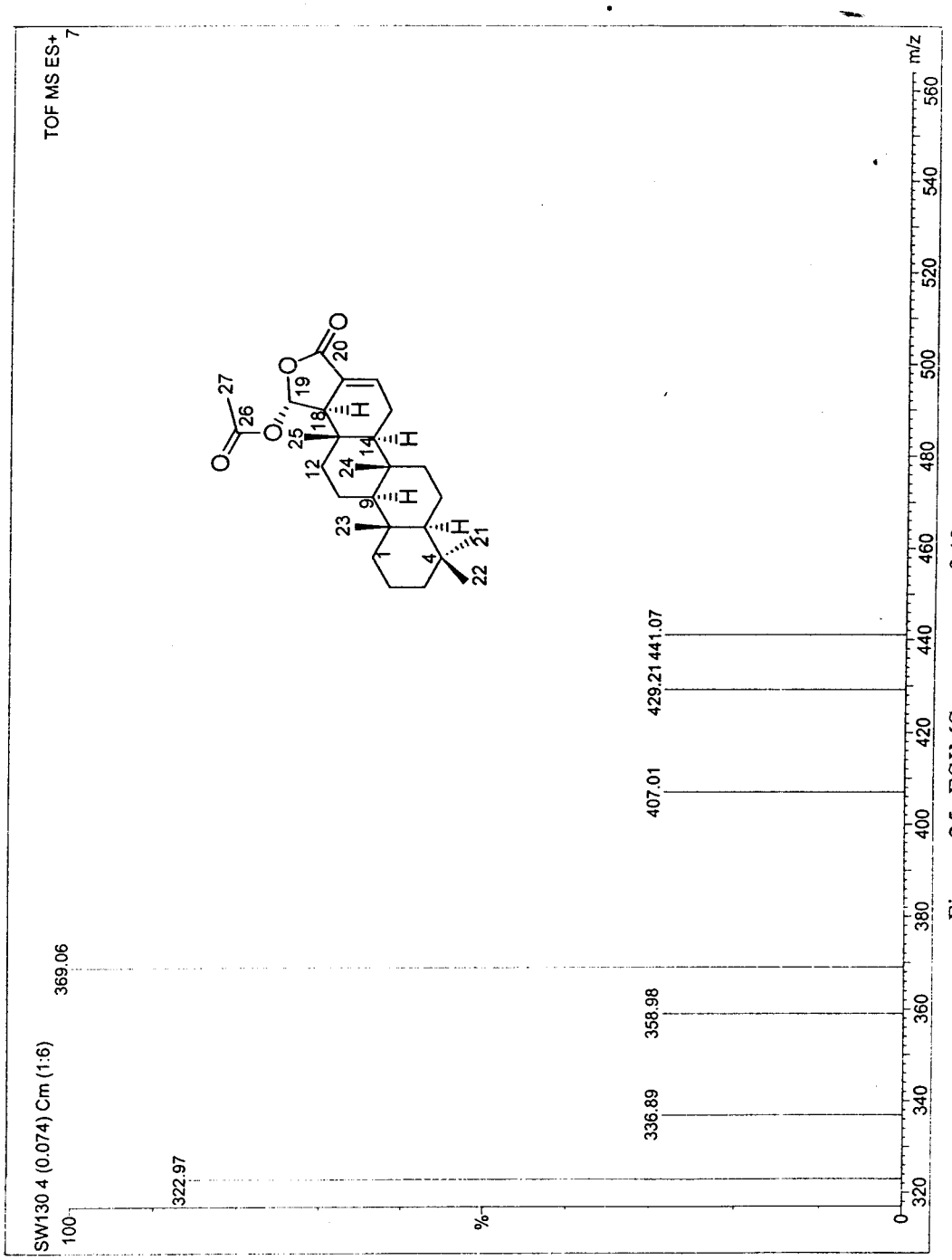

Figure 25 ESIMS spectrum of **40**

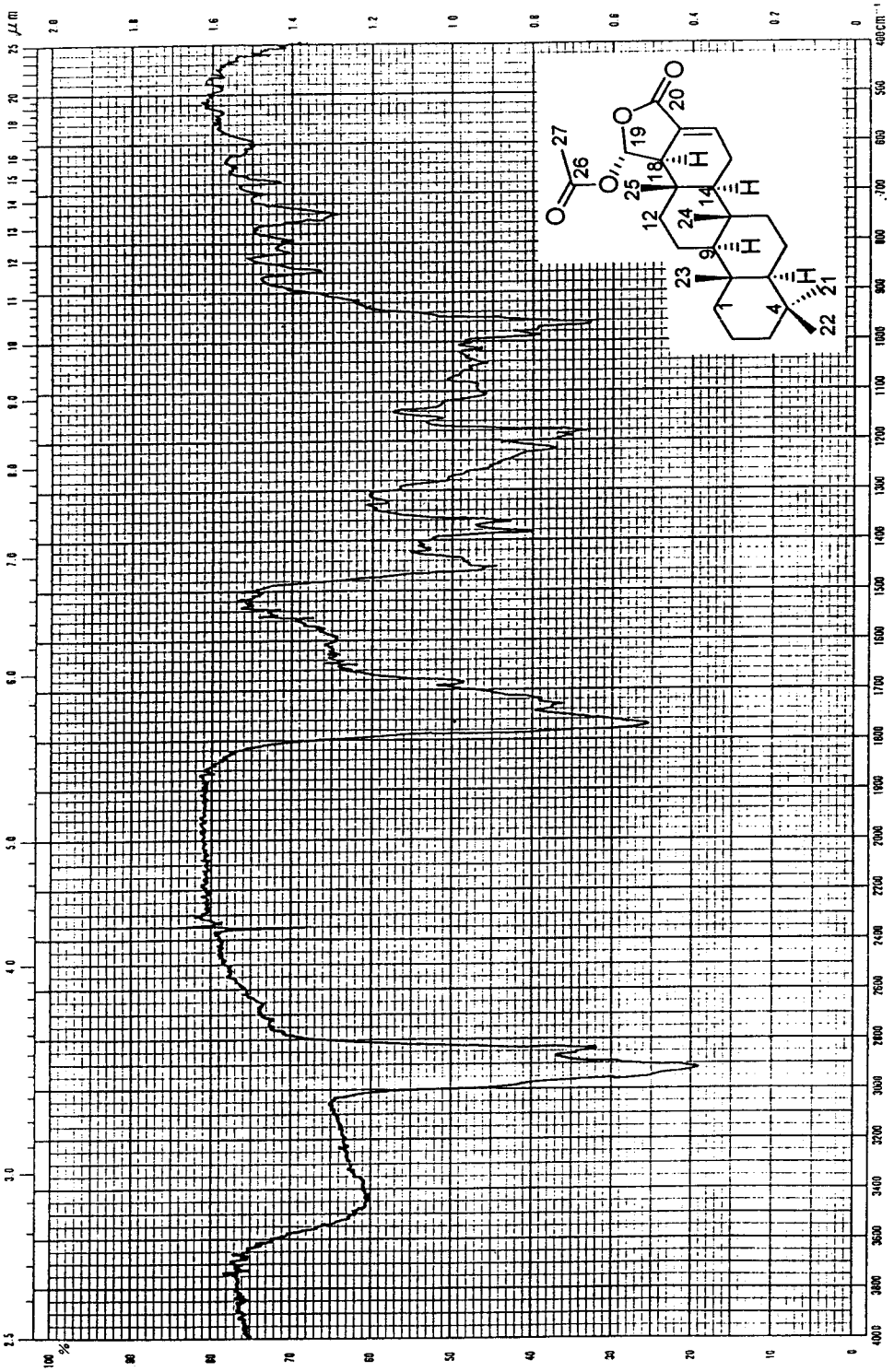

Figure 26 IR spectrum of **40** (thin film)

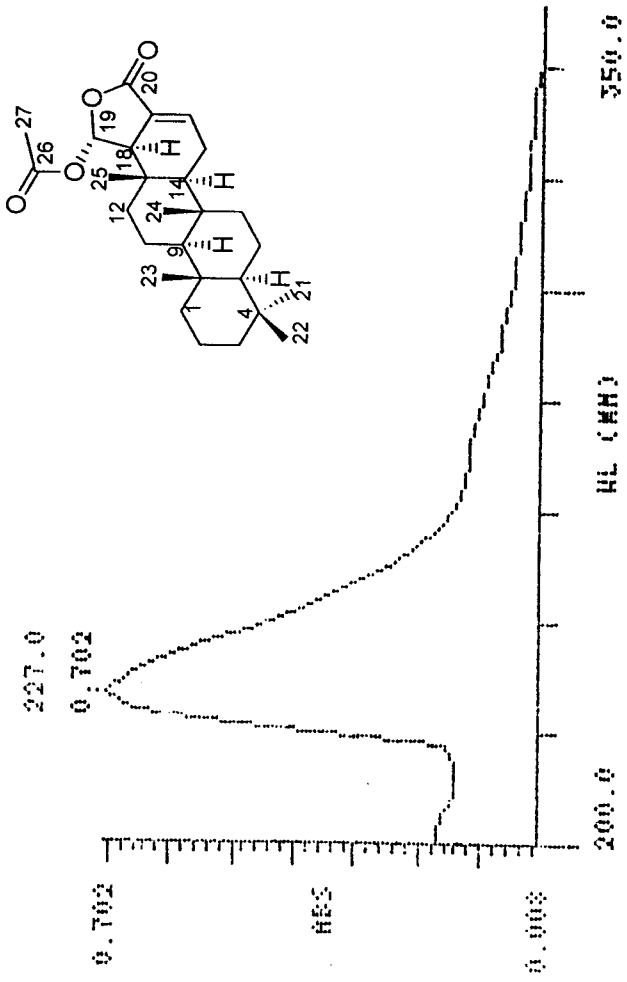

Figure 27 UV spectrum of **40** (MeOH)

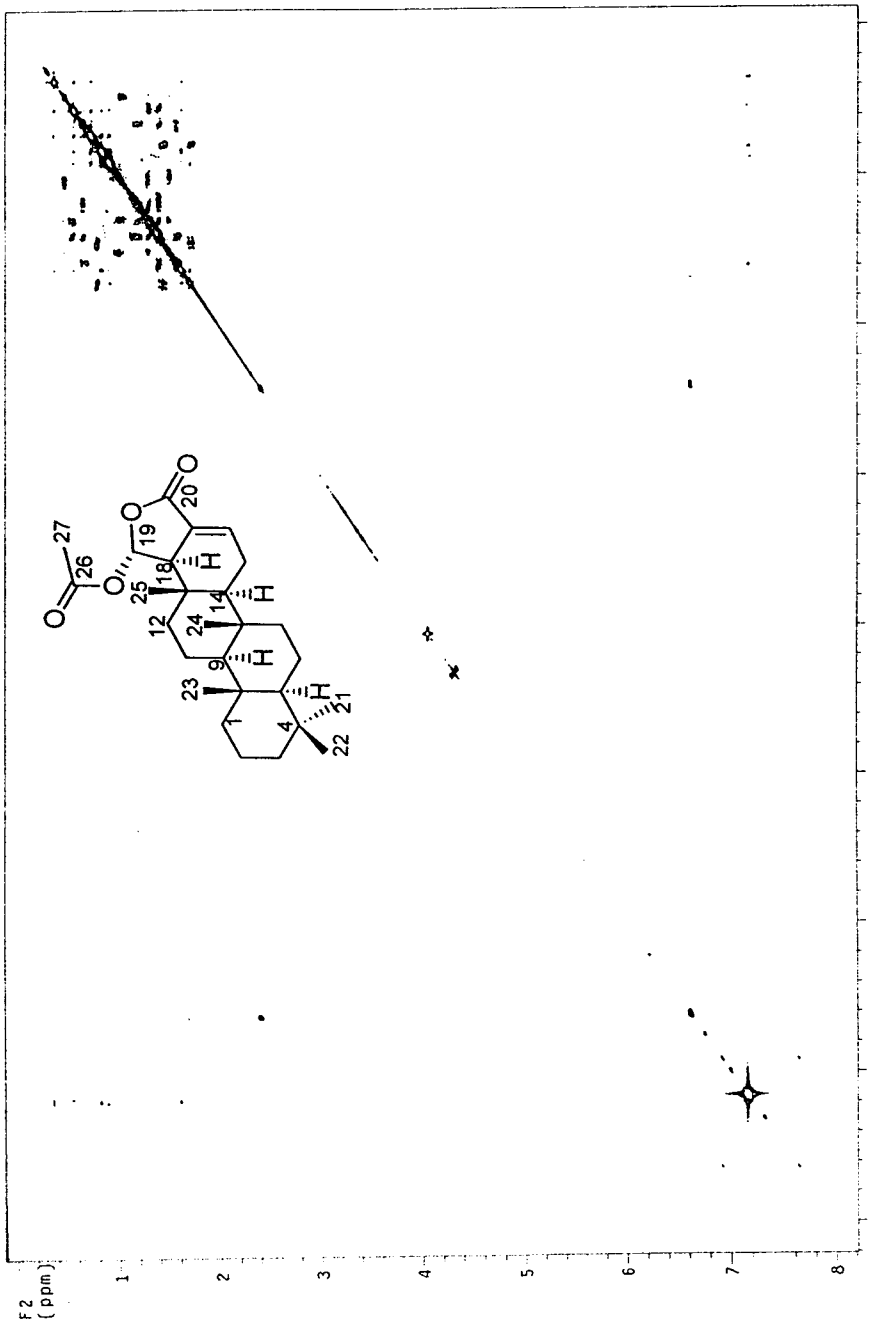

Figure 28 ^{1}H-^{1}H COSY spectrum of **40** (500 MHz; C_6D_6)

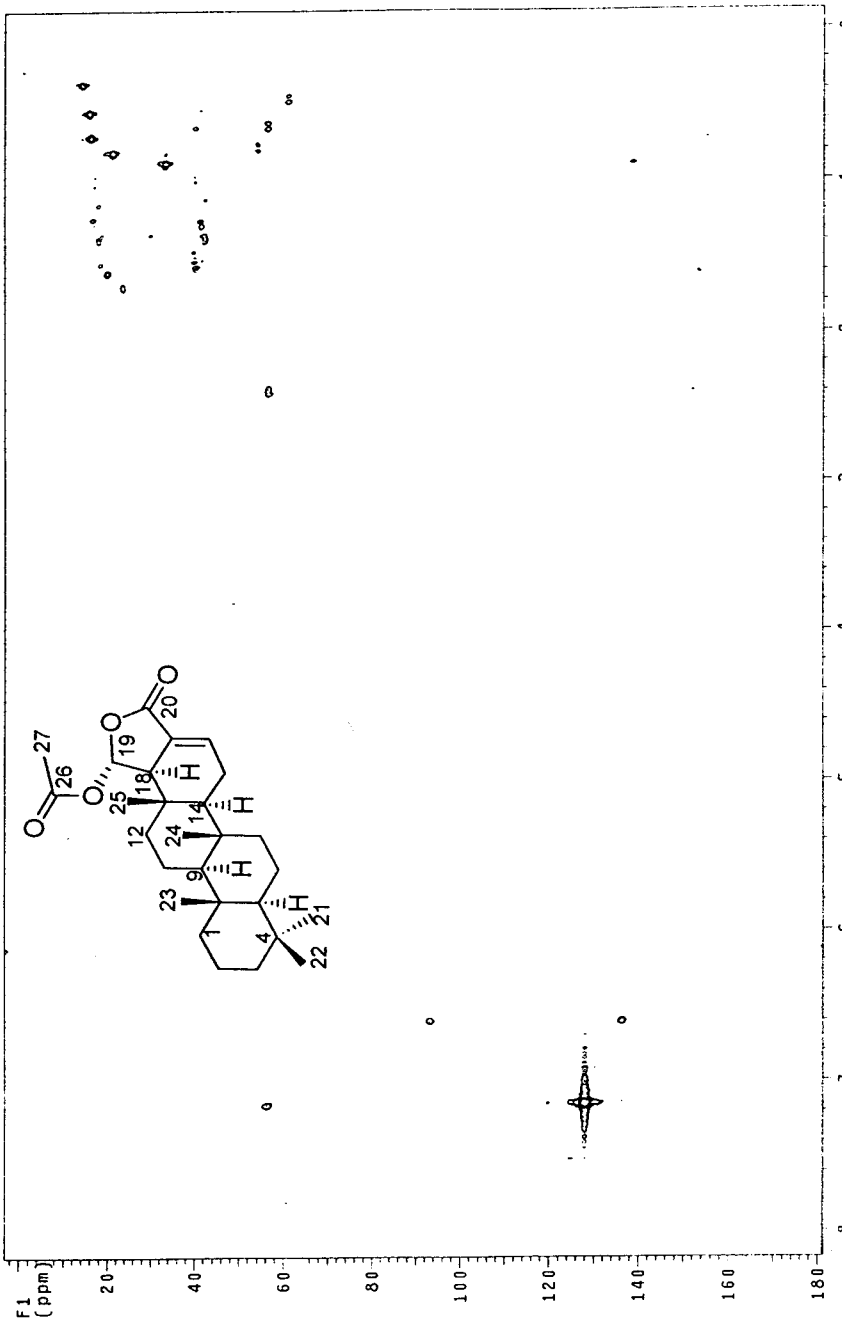

Figure 29 HMQC spectrum of **40** (500 MHz; C_6D_6)

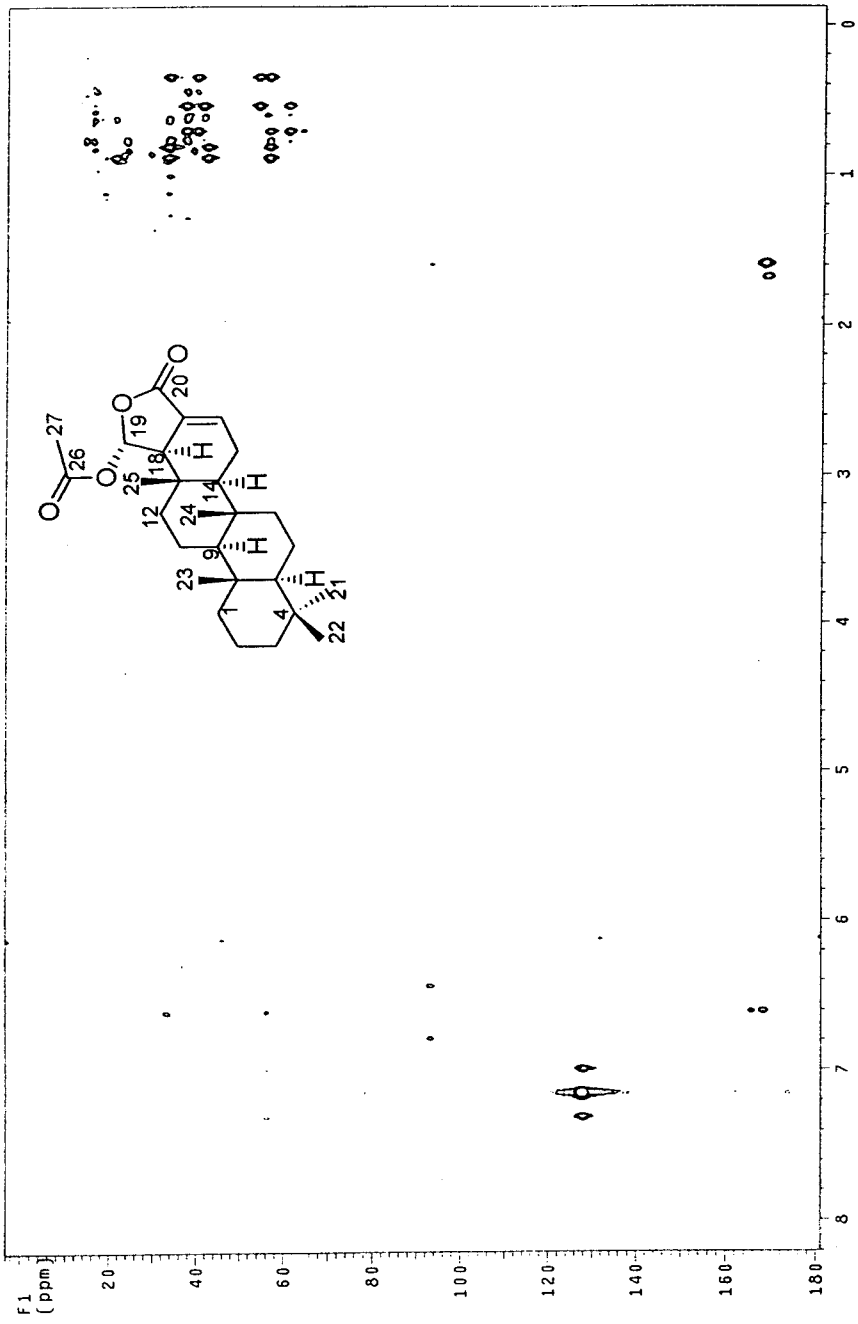

Figure 30 HMBC spectrum of **40** (500 MHz; C_6D_6)

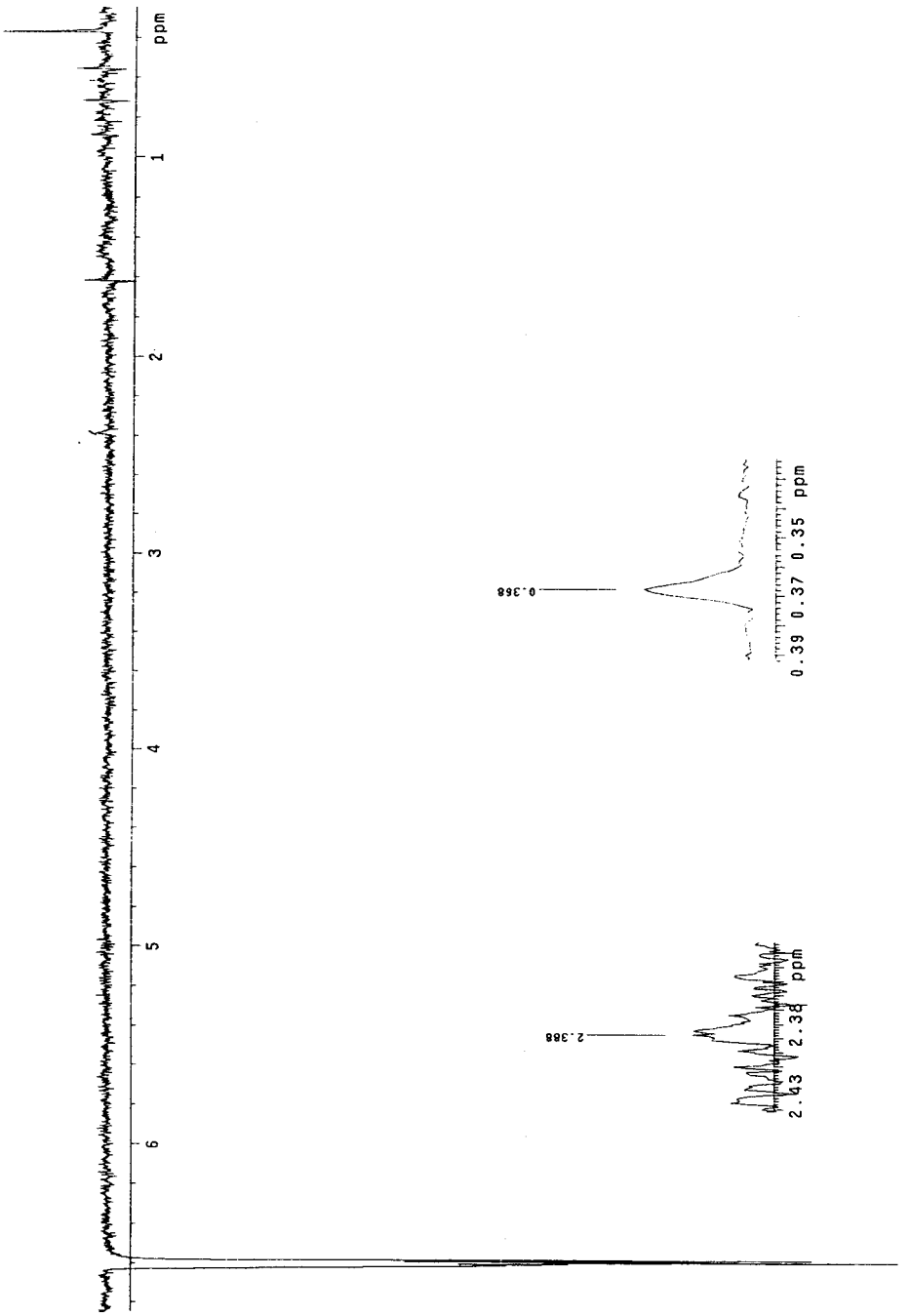

Figure 31 nOe difference spectrum of **40** after irradiation at δ_H 6.60 (H-19)

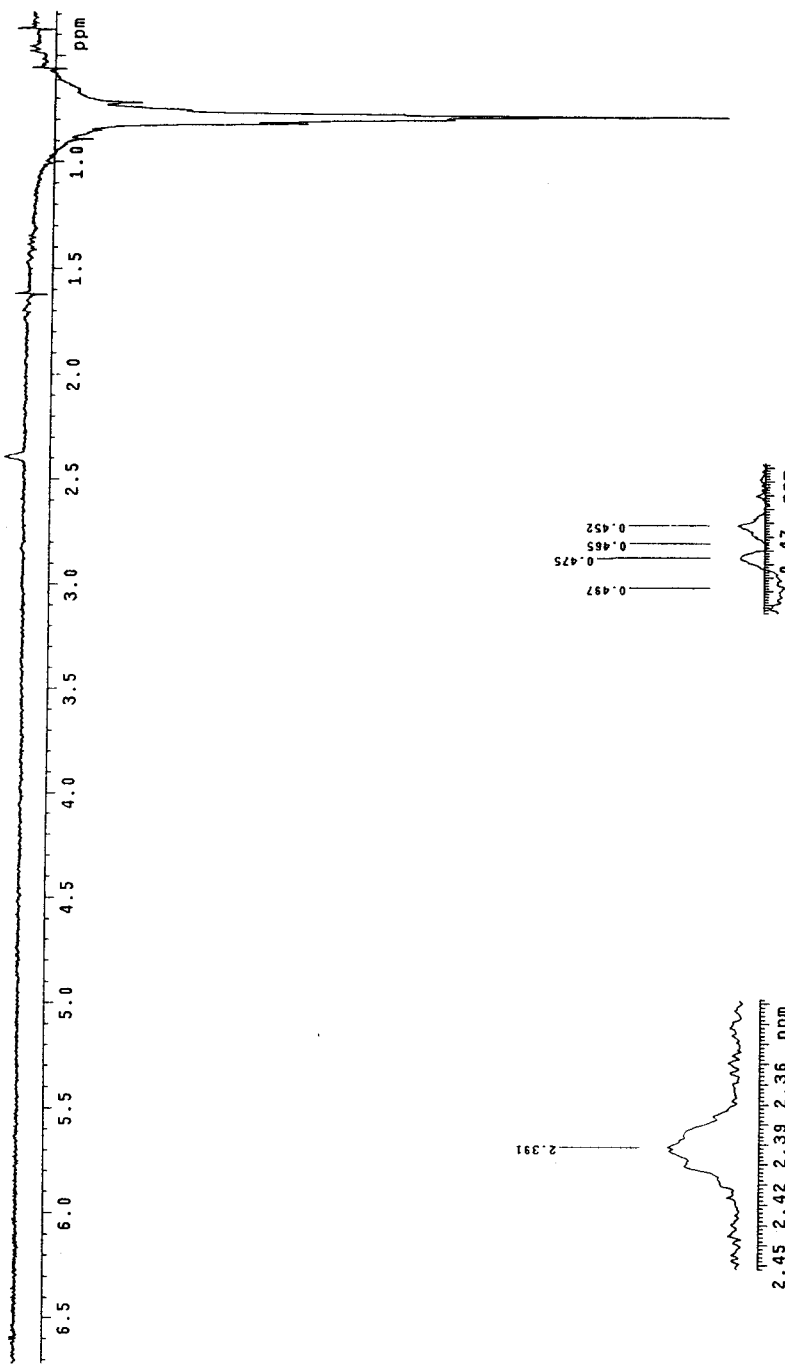

Figure 32 nOe difference spectrum of **40** after irradiation at δ_H 0.78 (H-14)

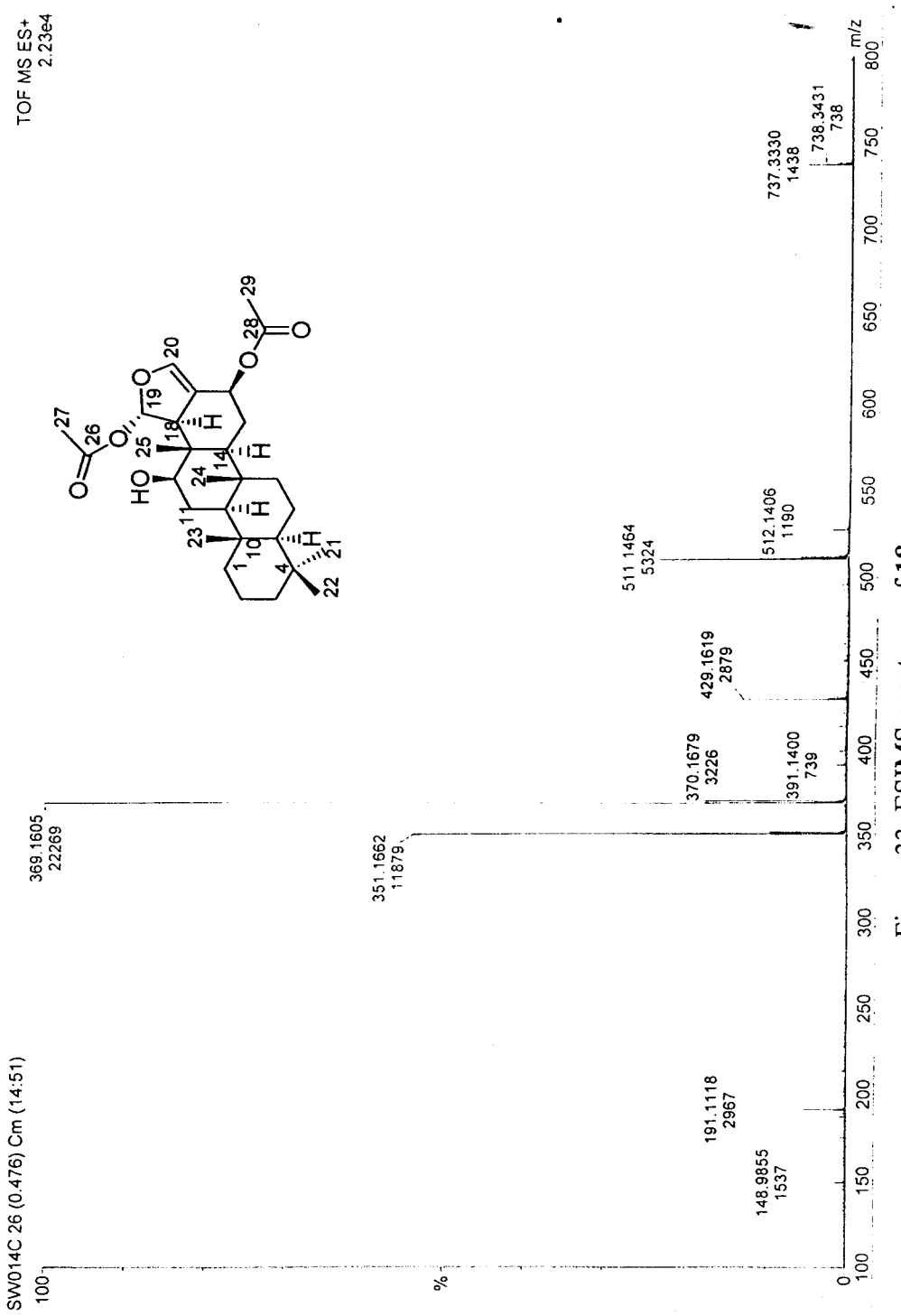

Figure 33 ESIMS spectrum of 18

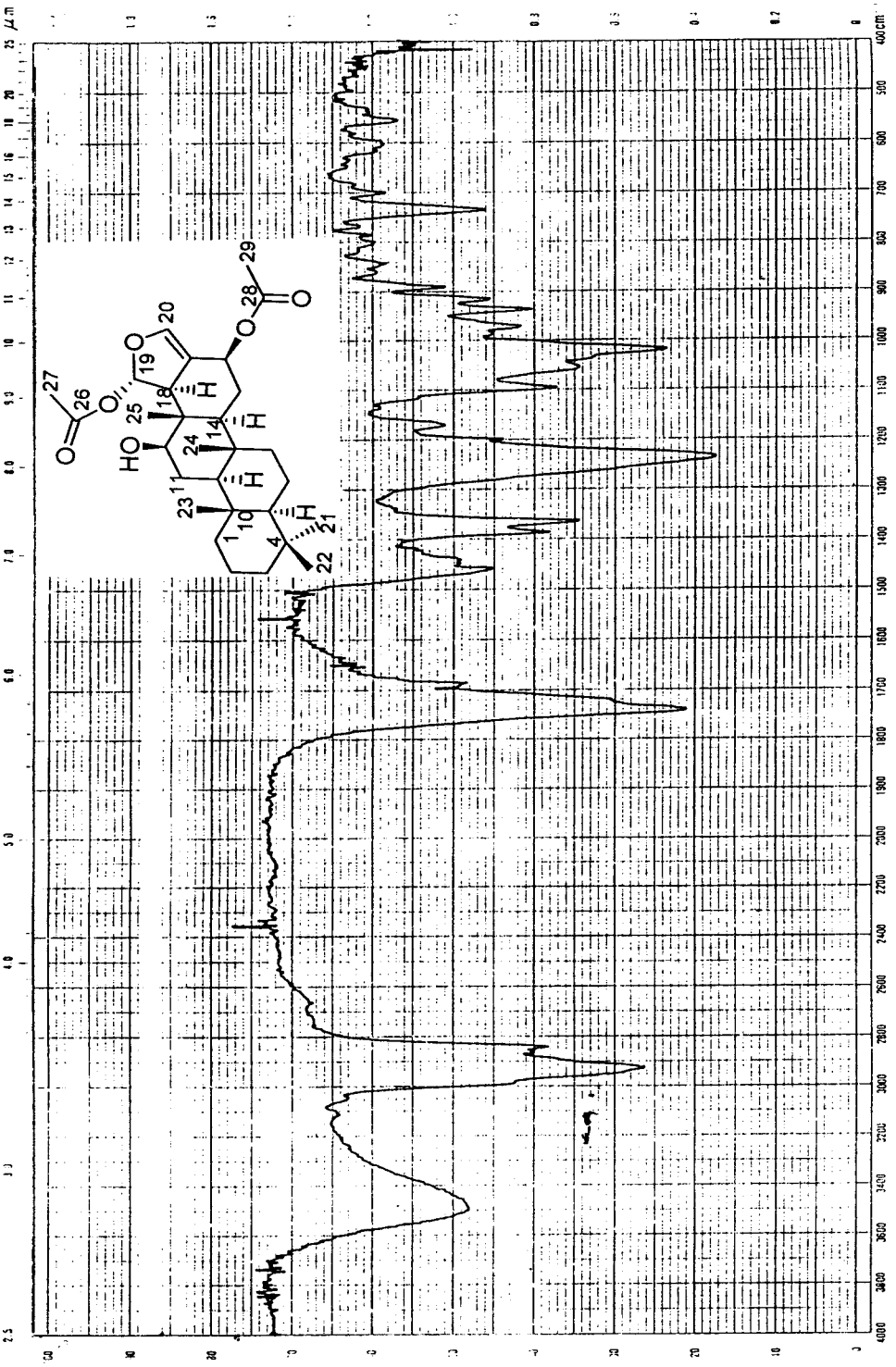

Figure 34 IR spectrum of **18** (thin film)

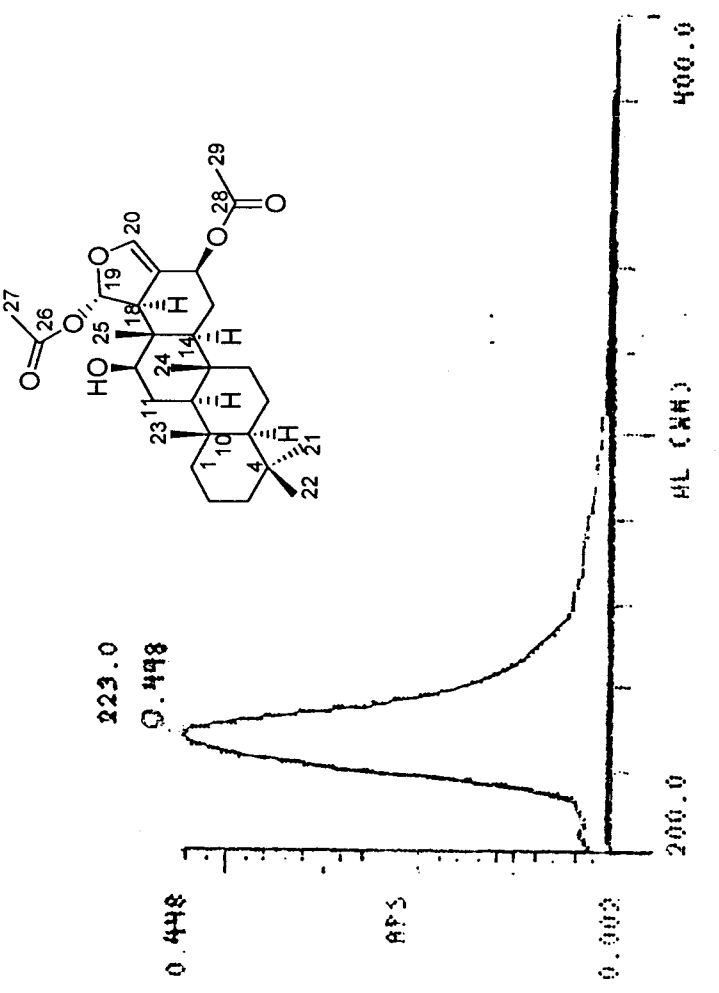

Figure 35 UV spectrum of **18** (MeOH)

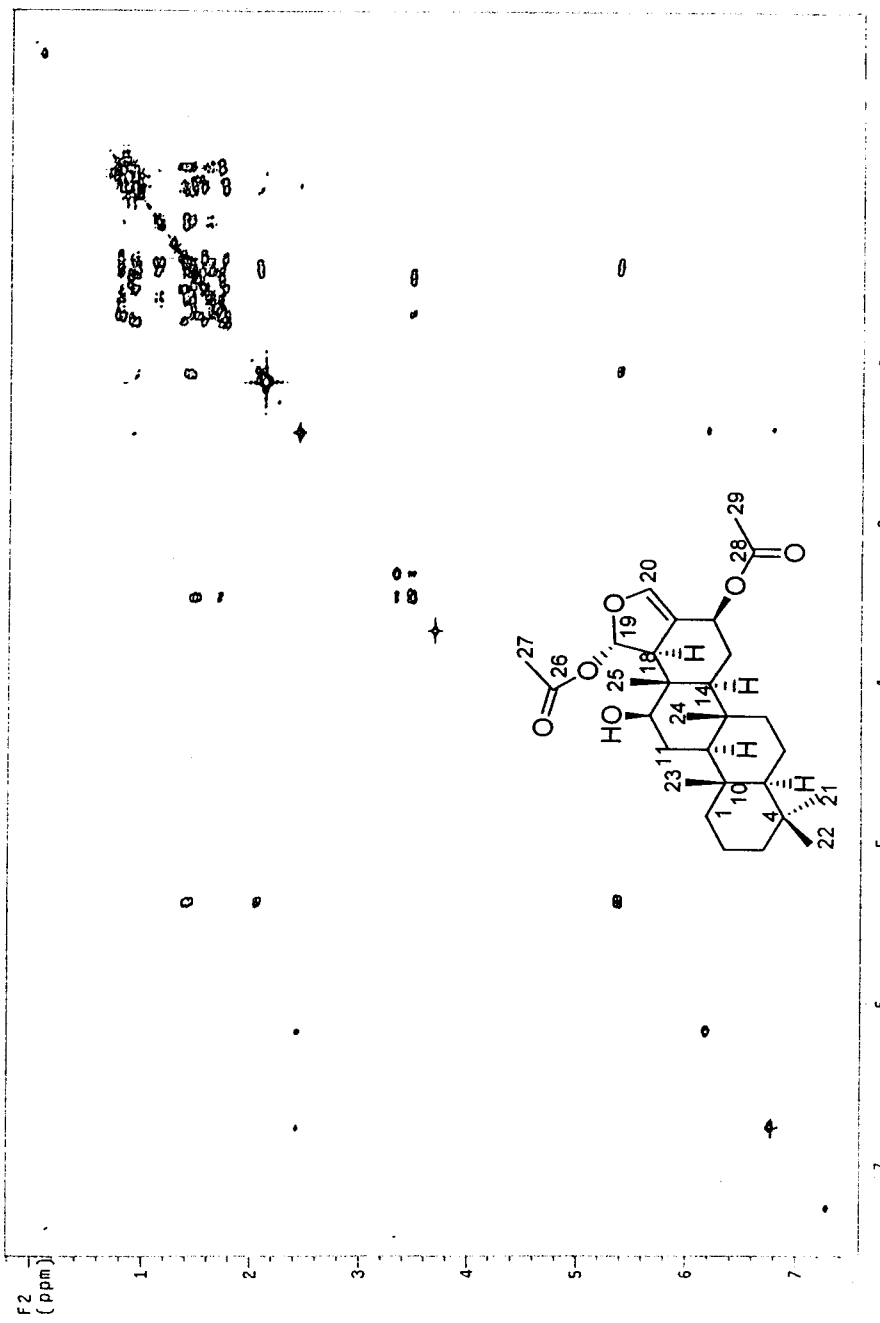

Figure 36 ^{1}H-^{1}H COSY spectrum of **18** (500 MHz; $CDCl_3$)

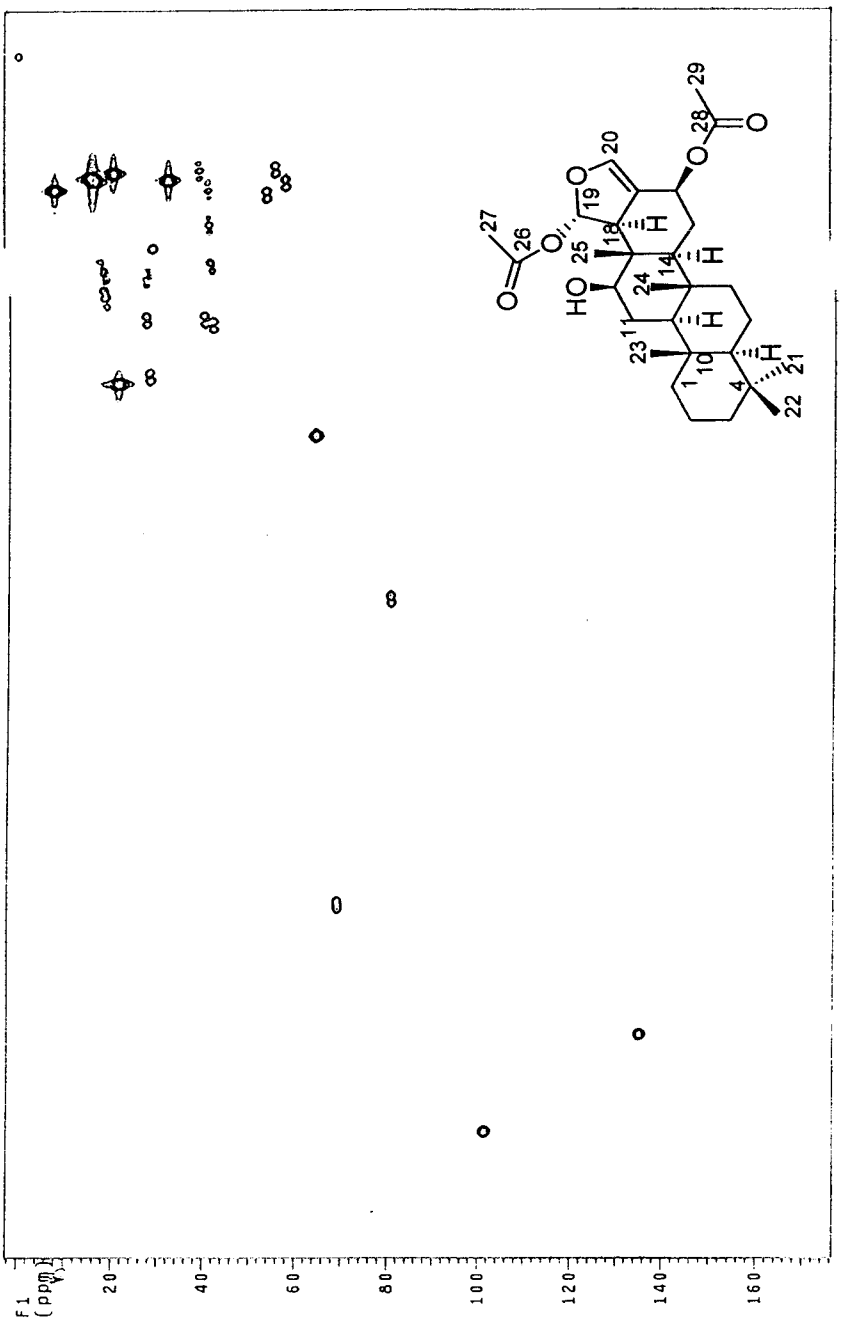

Figure 37 HMQC spectrum of **18** (500 MHz; $CDCl_3$)

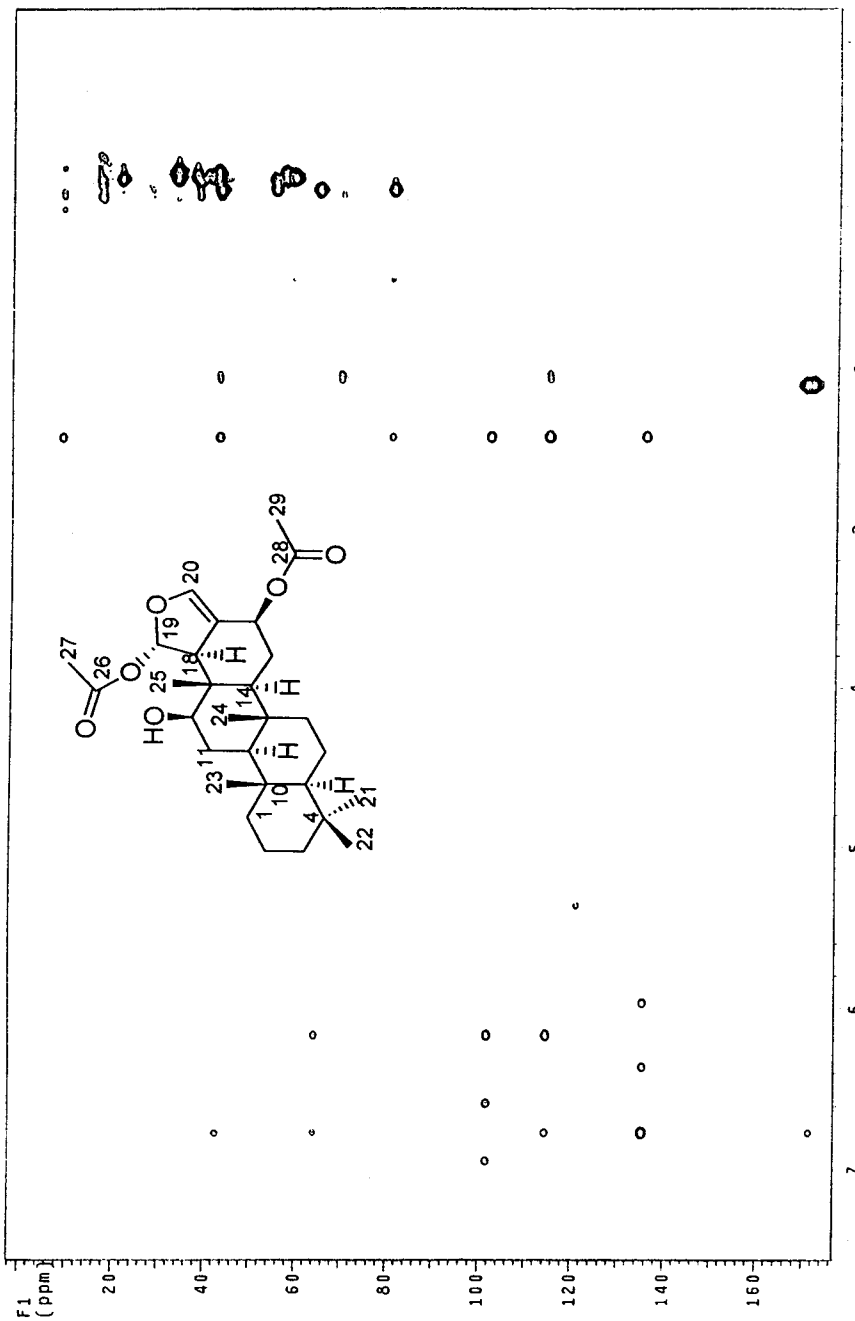

Figure 38 HMBC spectrum of **18** (500 MHz; $CDCl_3$)

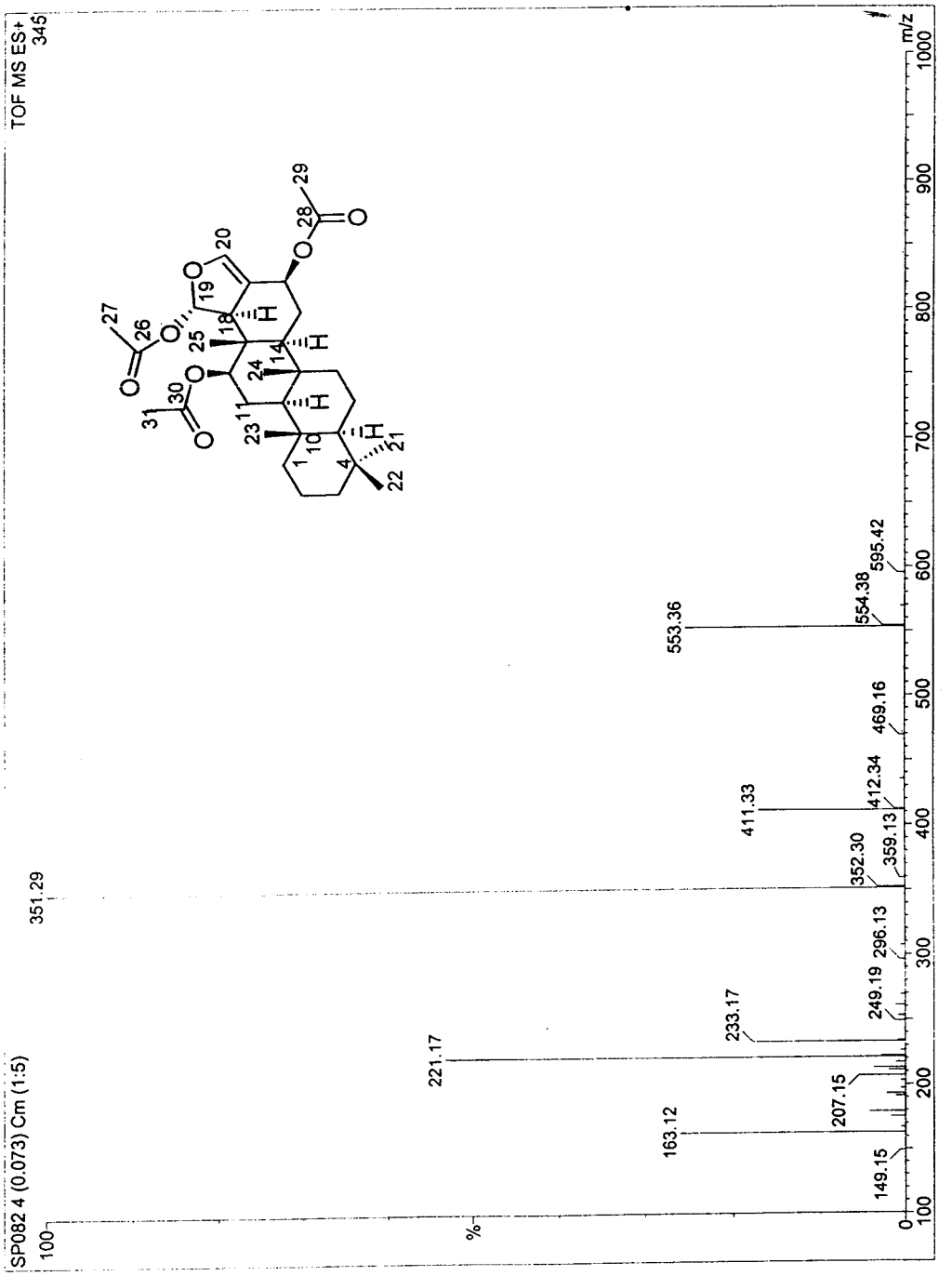

Figure 39 ESIMS spectrum of **41**

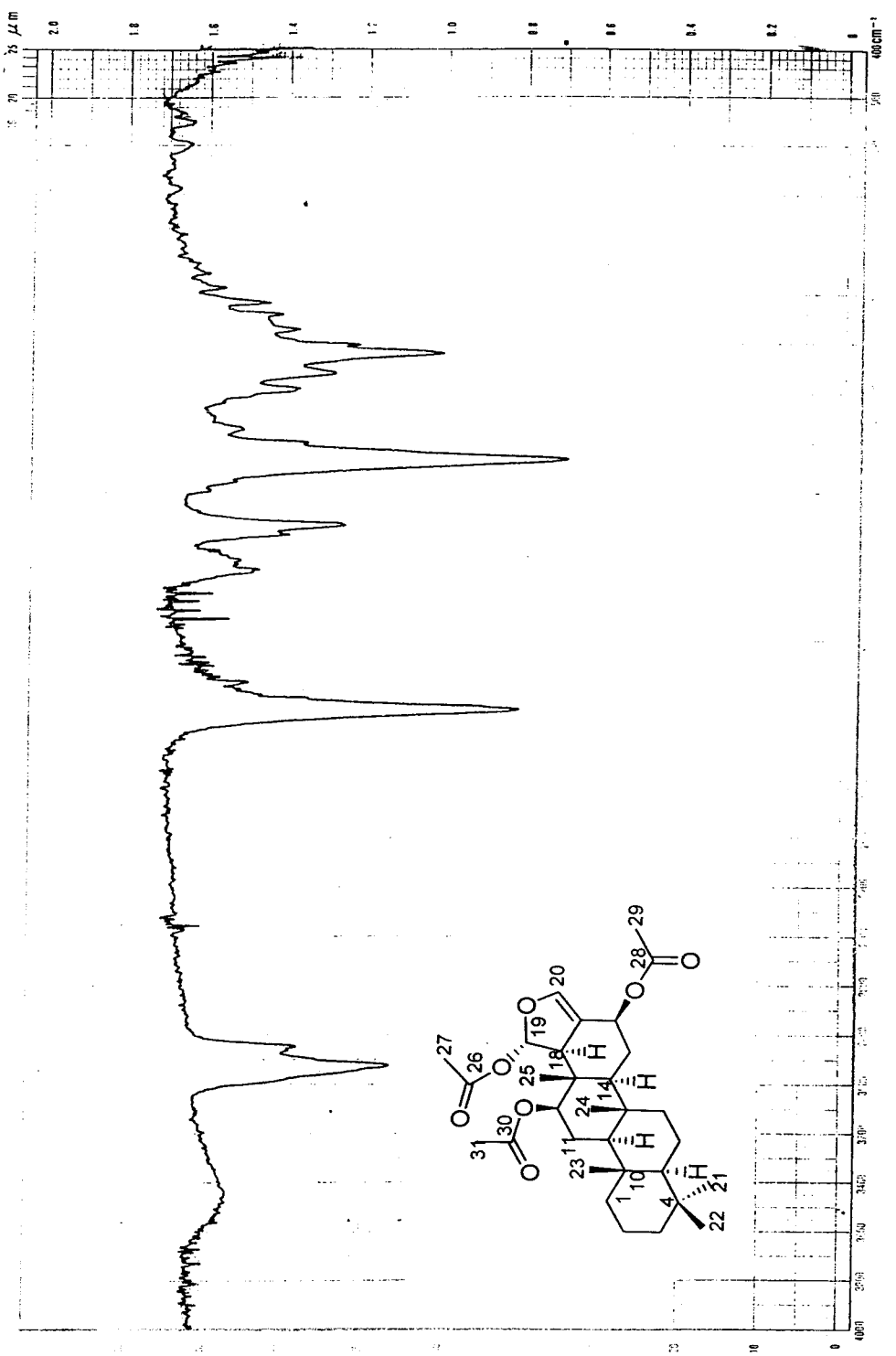

Figure 40 IR spectrum of **41** (thin film)

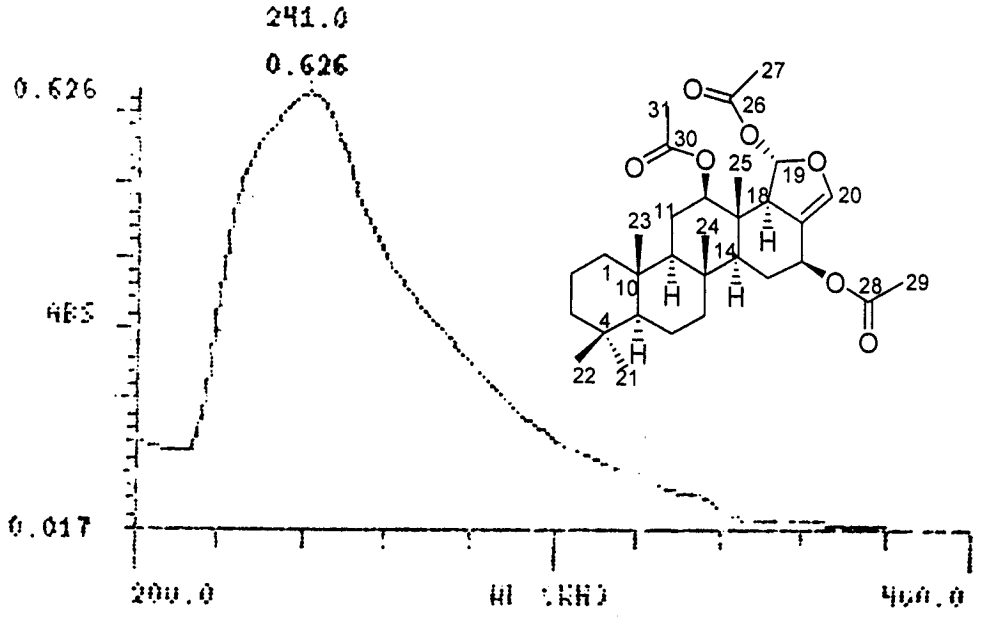

Figure 41 UV spectrum of **41** (MeOH)

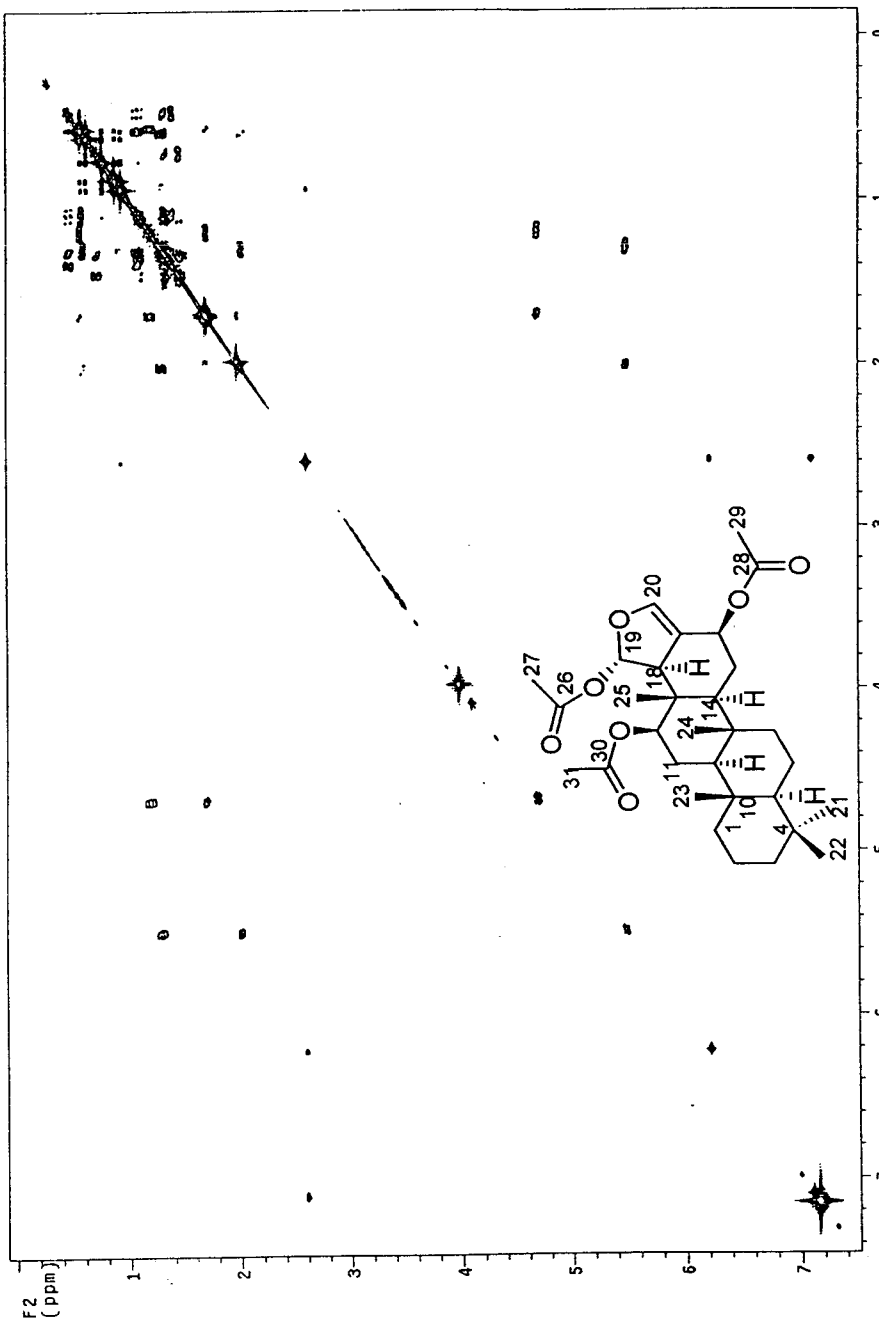

Figure 42 ^{1}H-^{1}H COSY spectrum of **41** (500 MHz; C_6D_6)

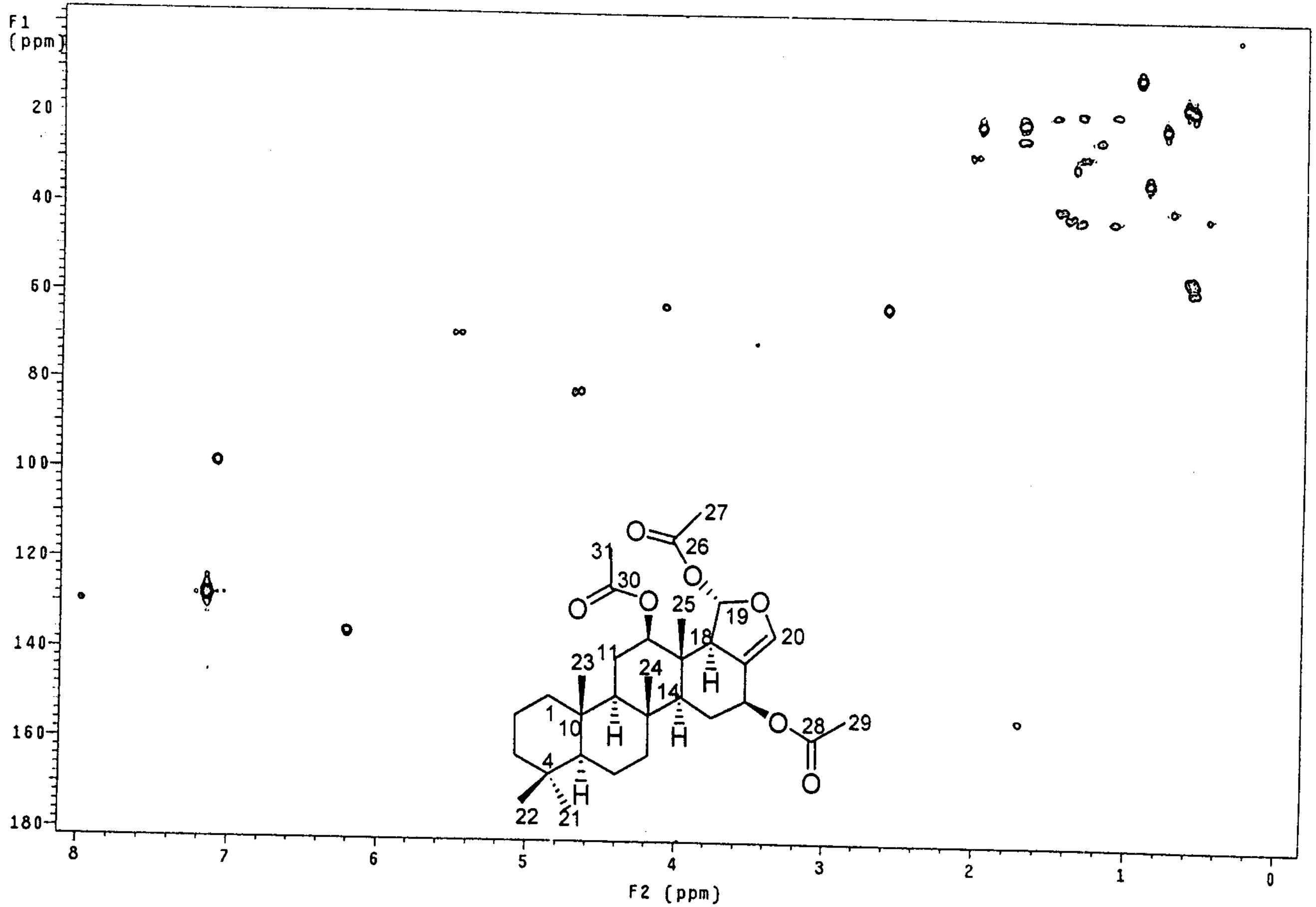

Figure 43 HMQC spectrum of **41** (500 MHz; C_6D_6)

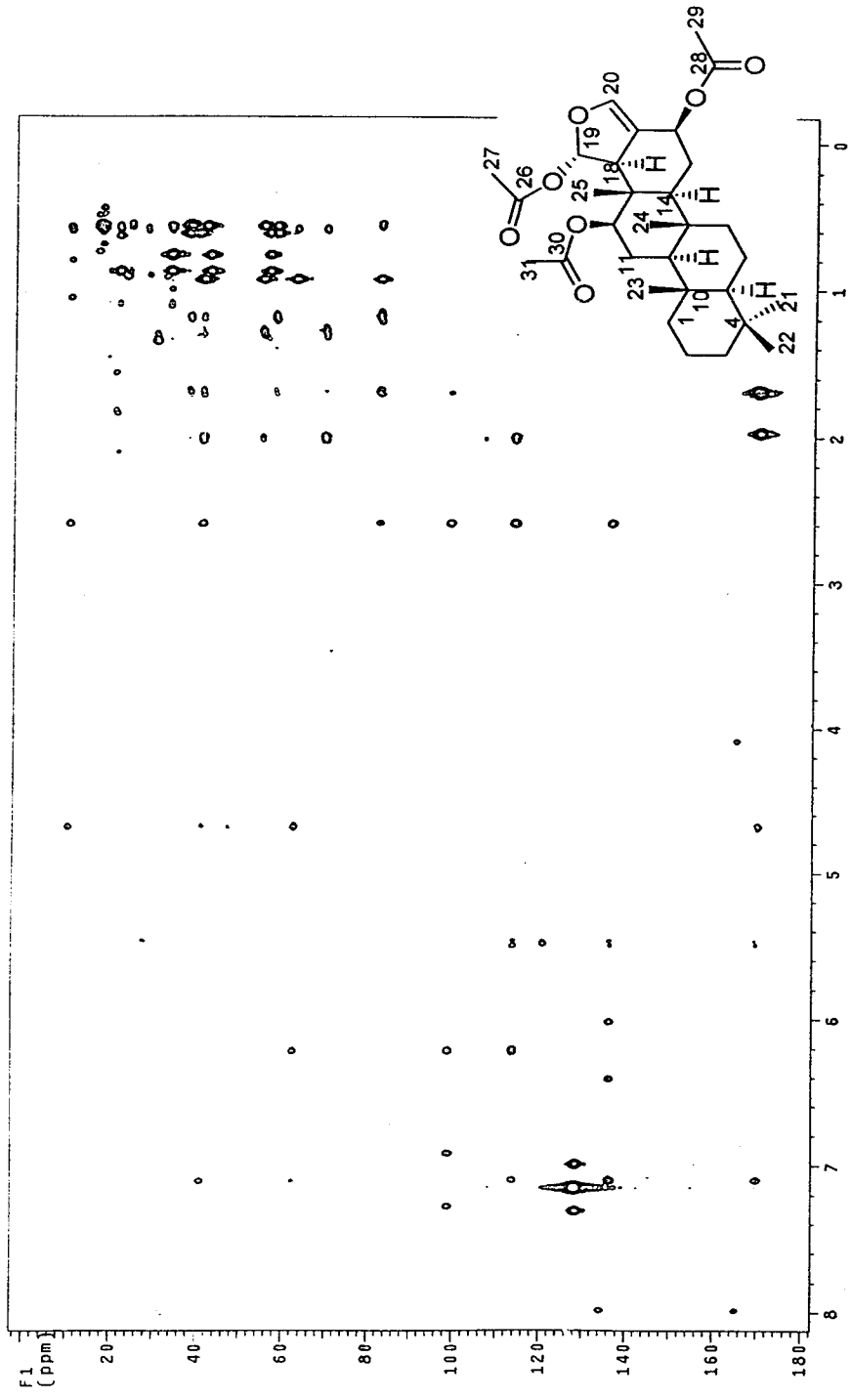

Figure 44 HMBC spectrum of **41** (500 MHz; C_6D_6)

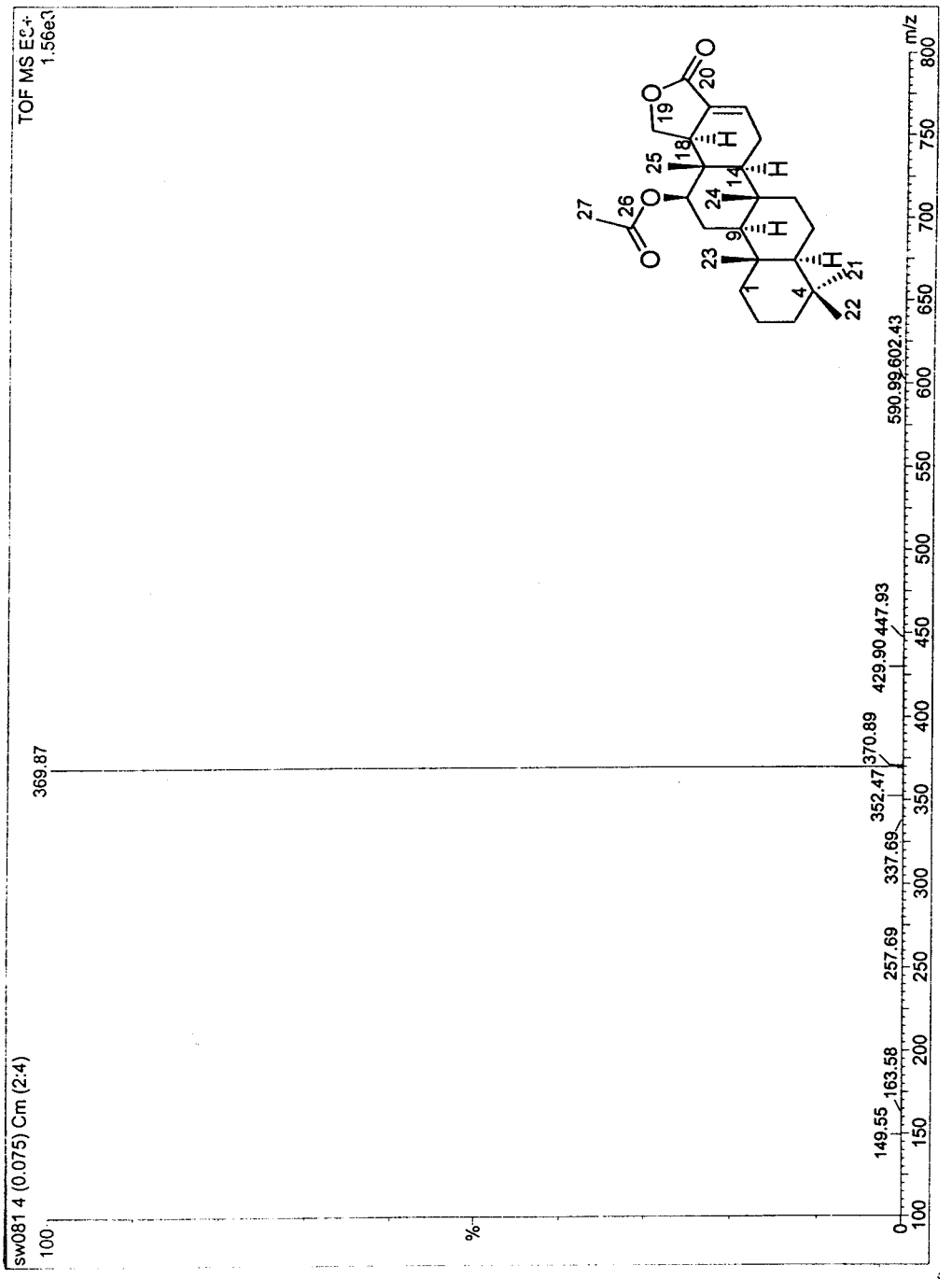

Figure 45 ESIMS spectrum of **42**

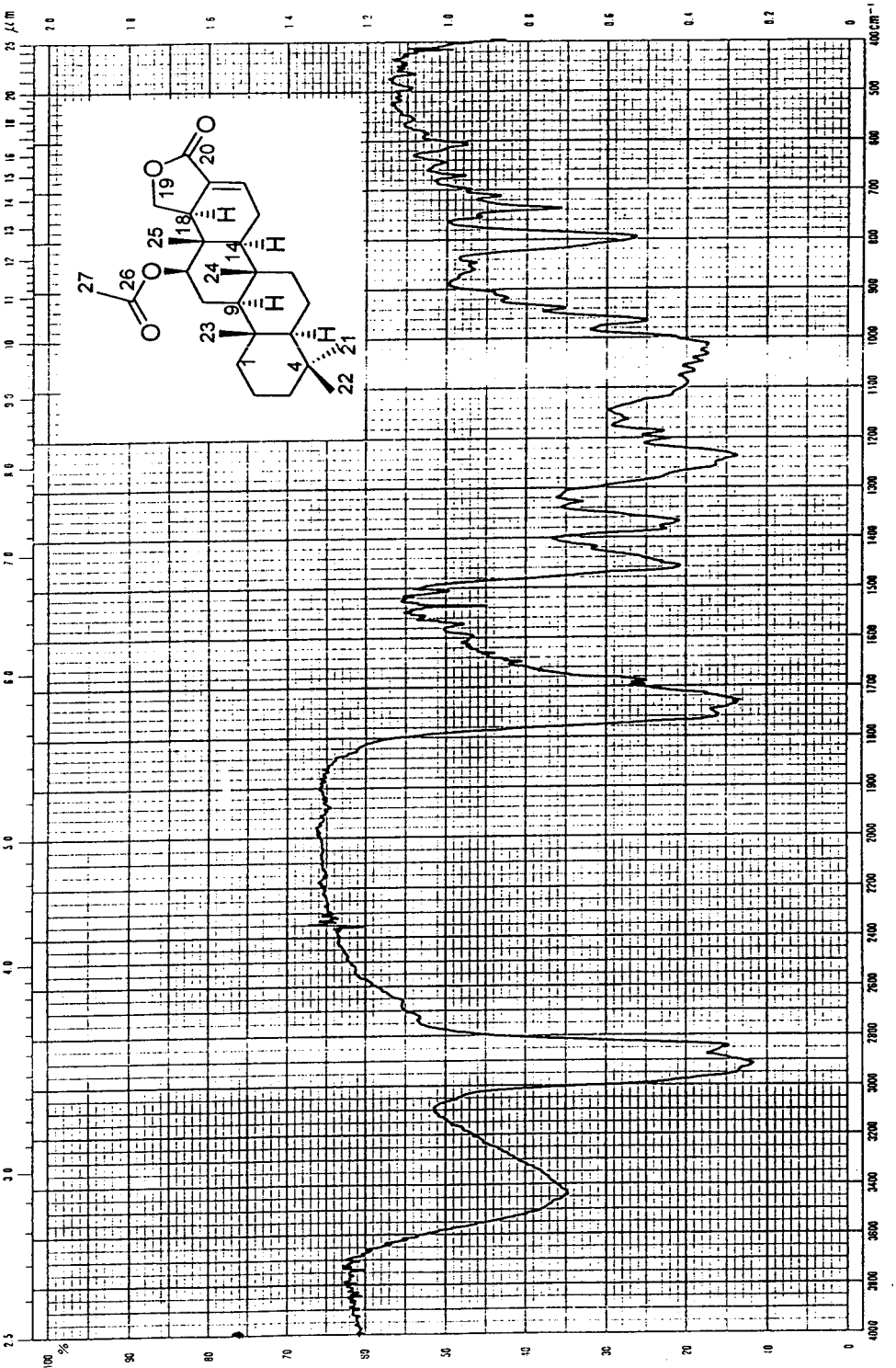

Figure 46 IR spectrum of **42** (thin film)

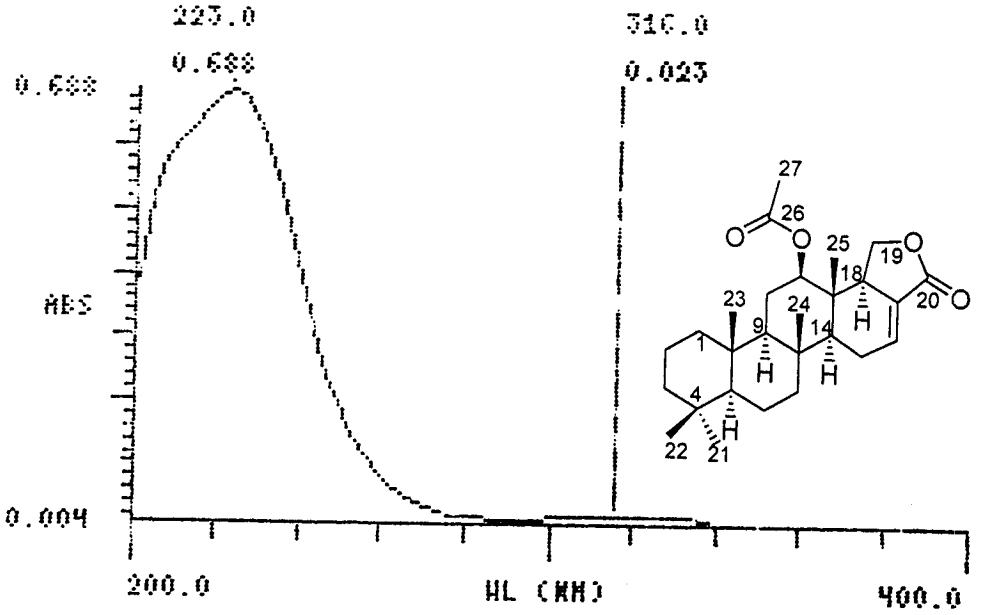

Figure 47 UV spectrum of **42** (MeOH)

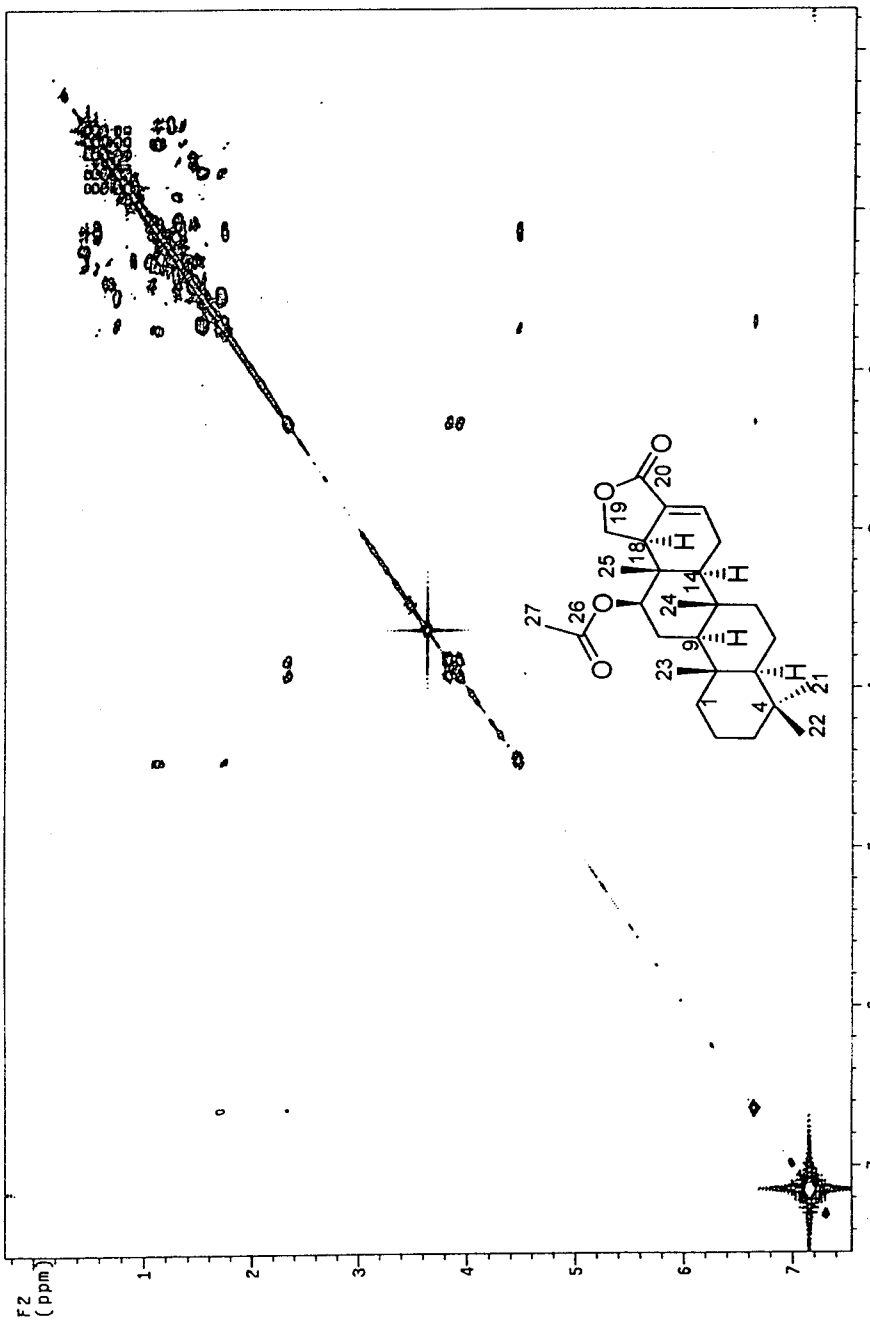

Figure 48 ^{1}H-^{1}H COSY spectrum of **42** (500 MHz; C_6D_6)

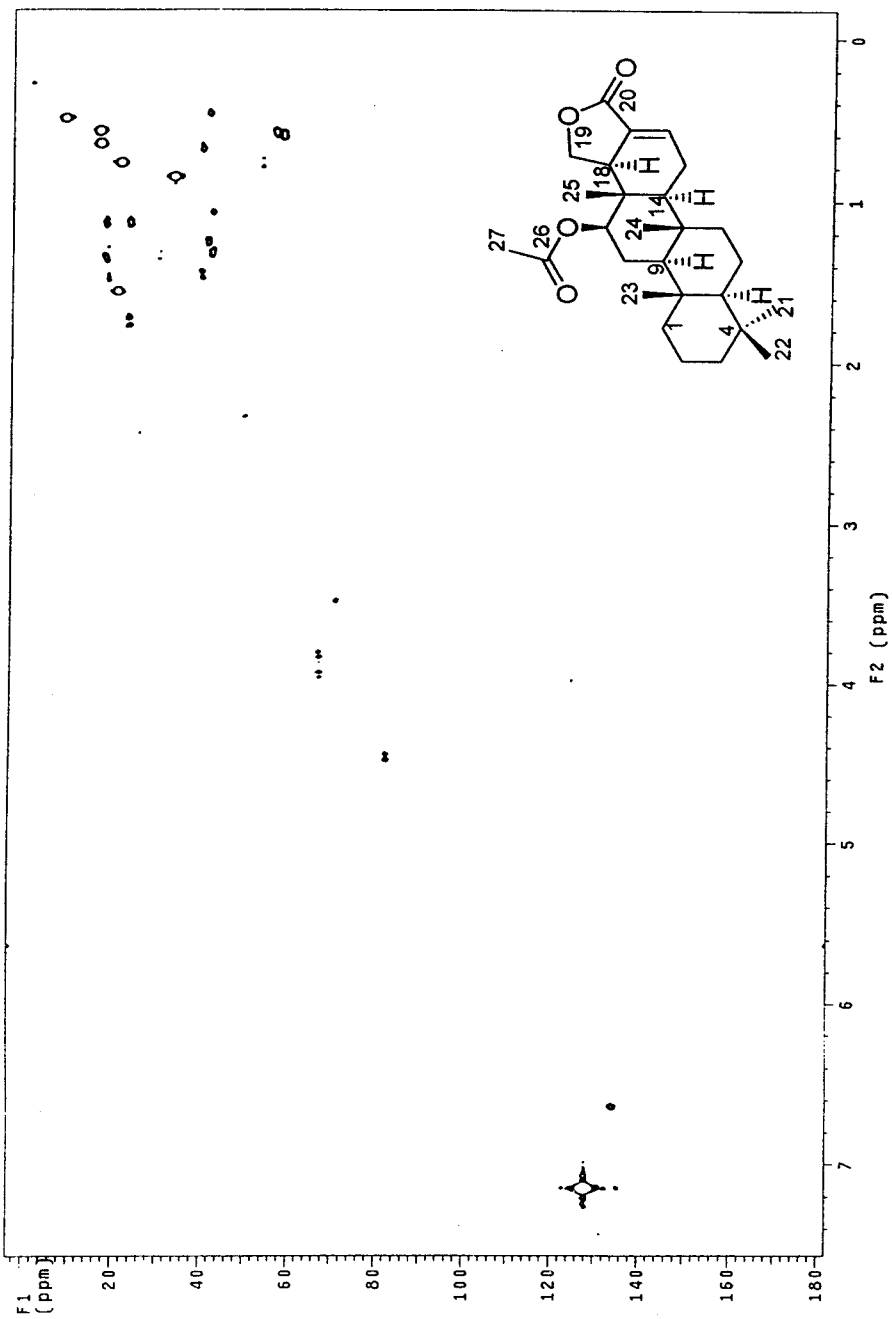

Figure 49 HMQC spectrum of **42** (500 MHz; C_6D_6)

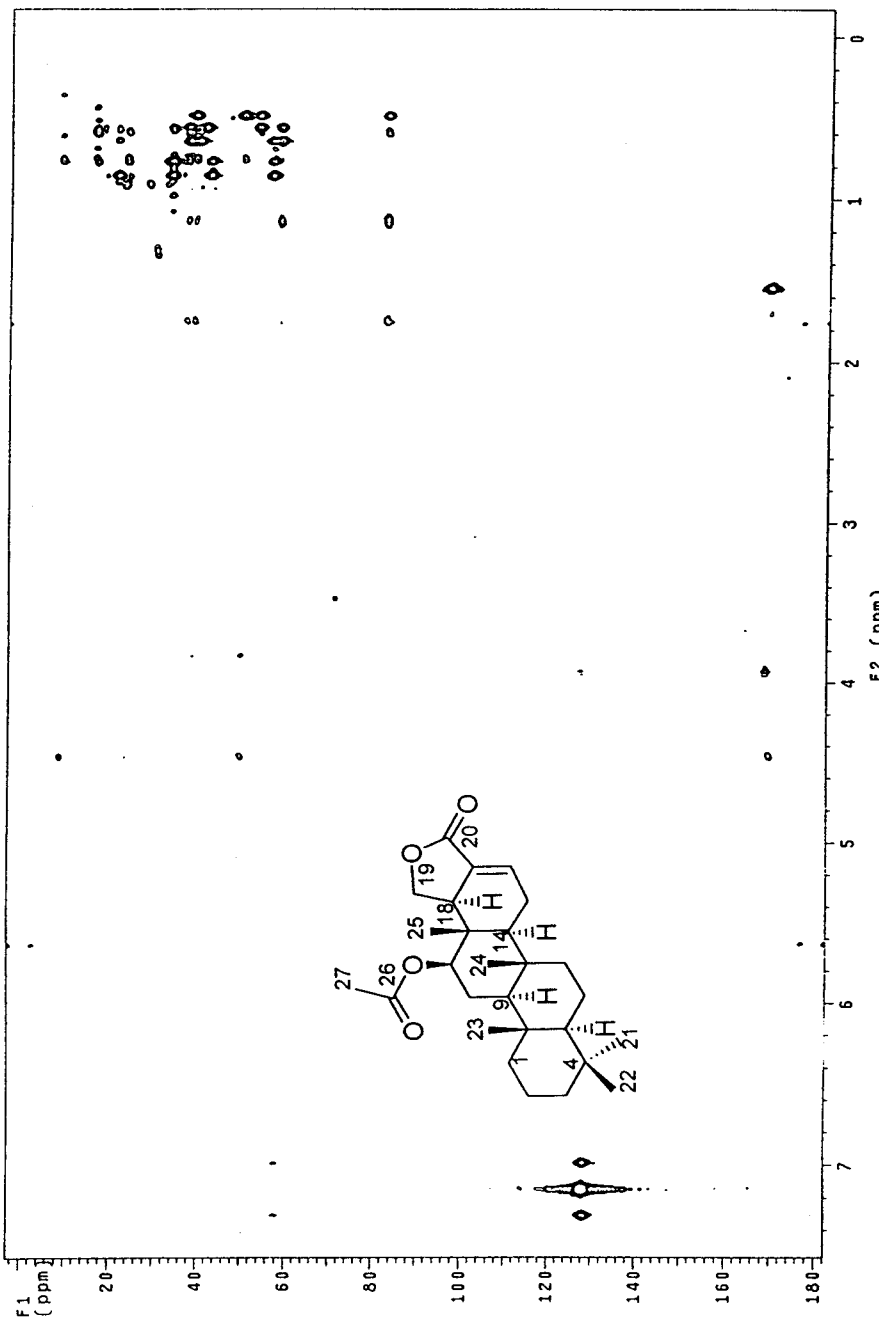

Figure 50 HMBC spectrum of **42** (500 MHz; C_6D_6)

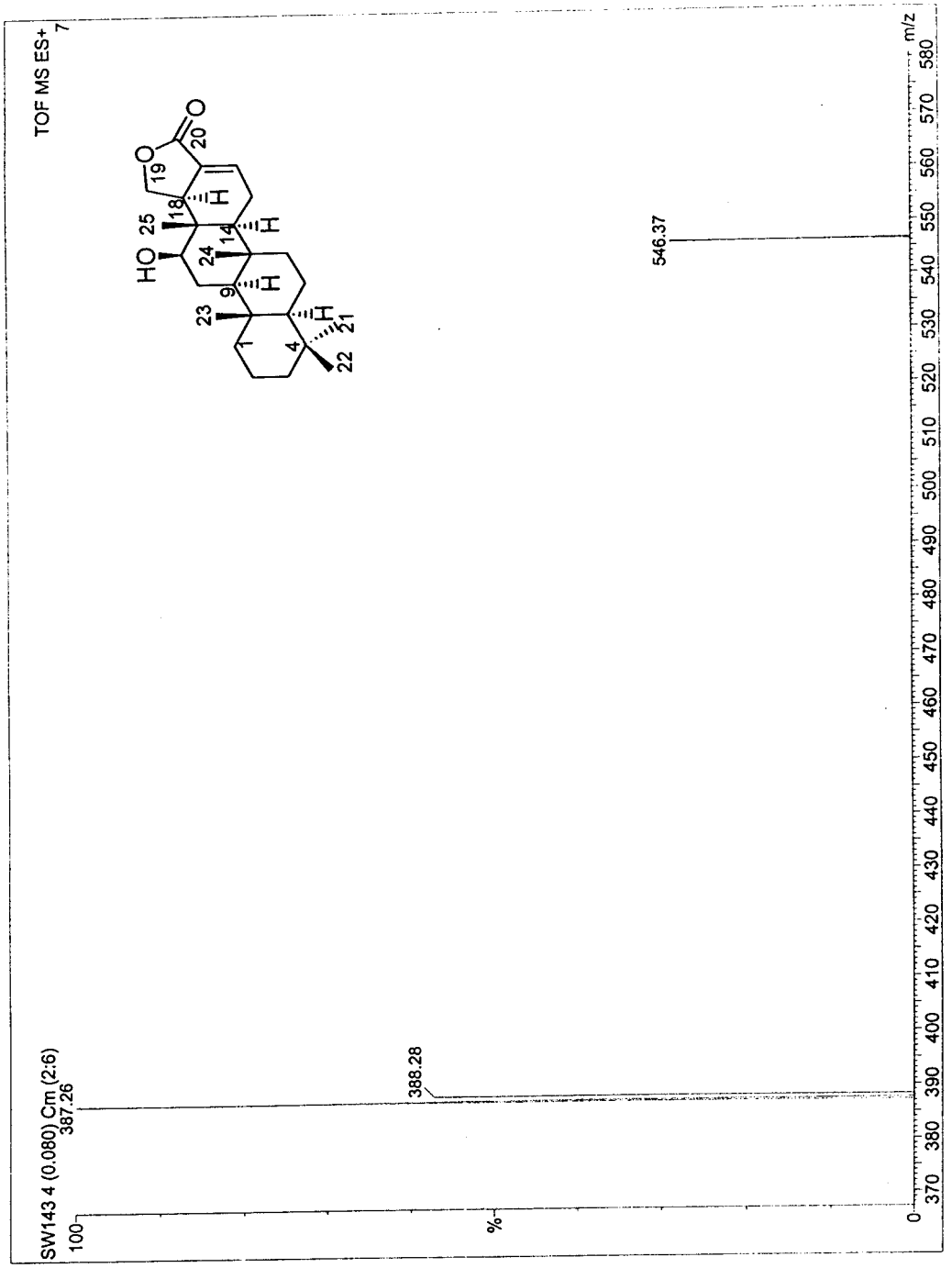

Figure 51 ESIMS spectrum of **43**

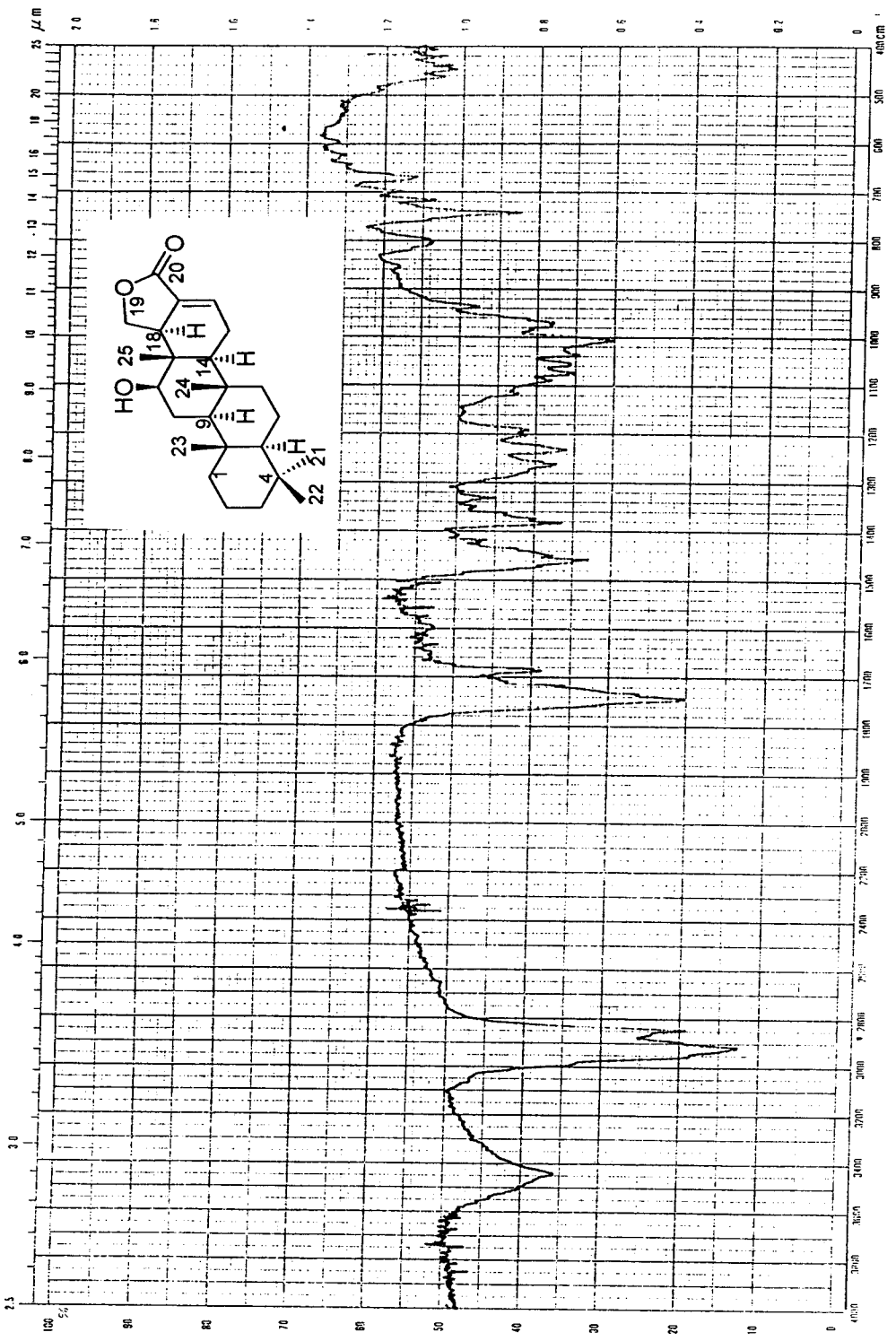

Figure 52 IR spectrum of **43** (thin film)

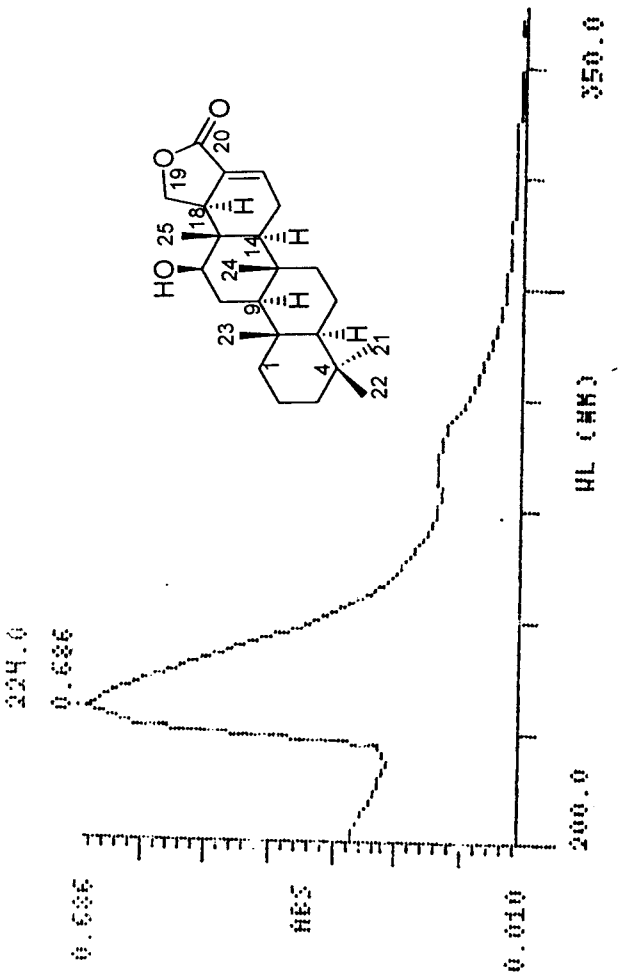

Figure 53 UV spectrum of **43** (MeOH)

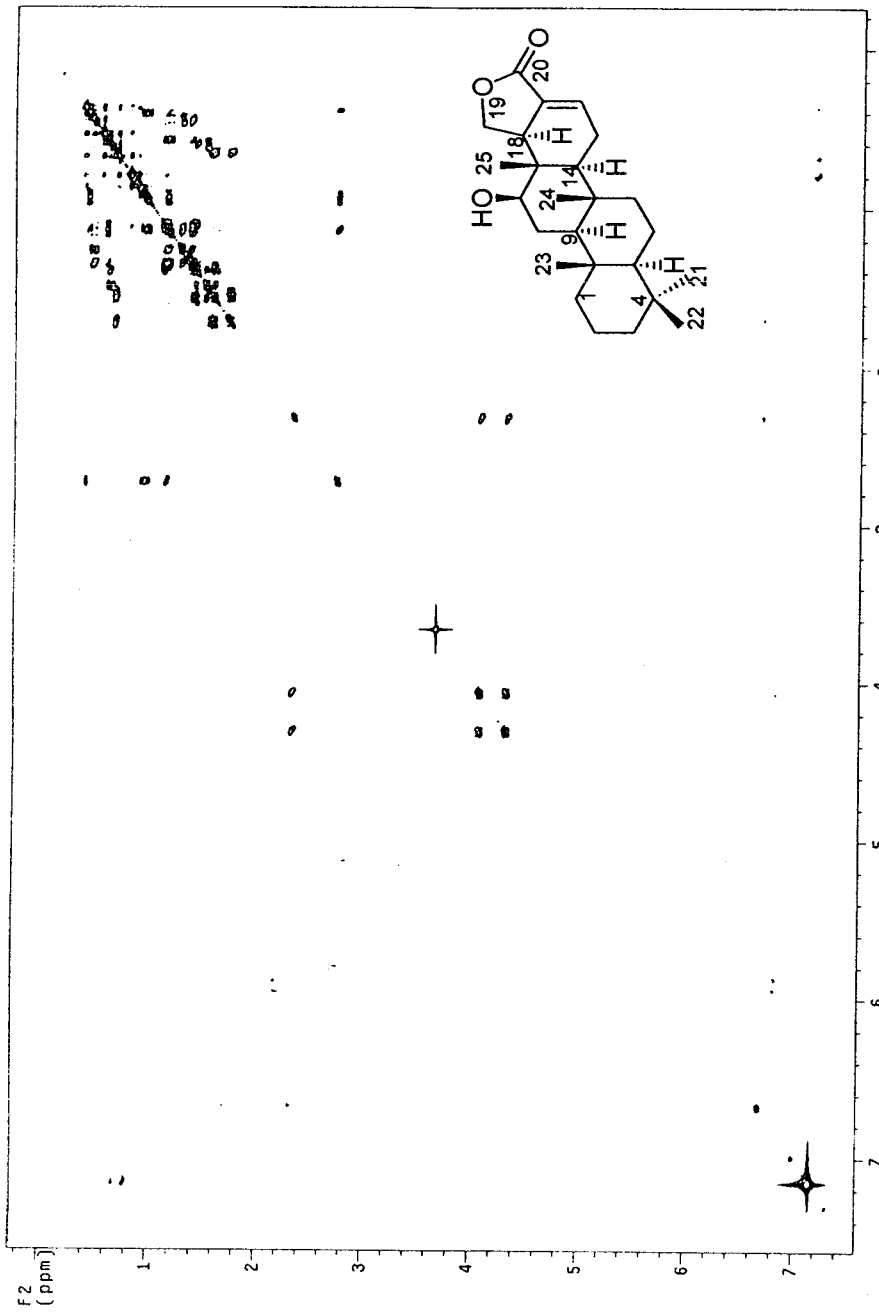

Figure 54 ^{1}H-^{1}H COSY spectrum of **43** (500 MHz; C_6D_6)

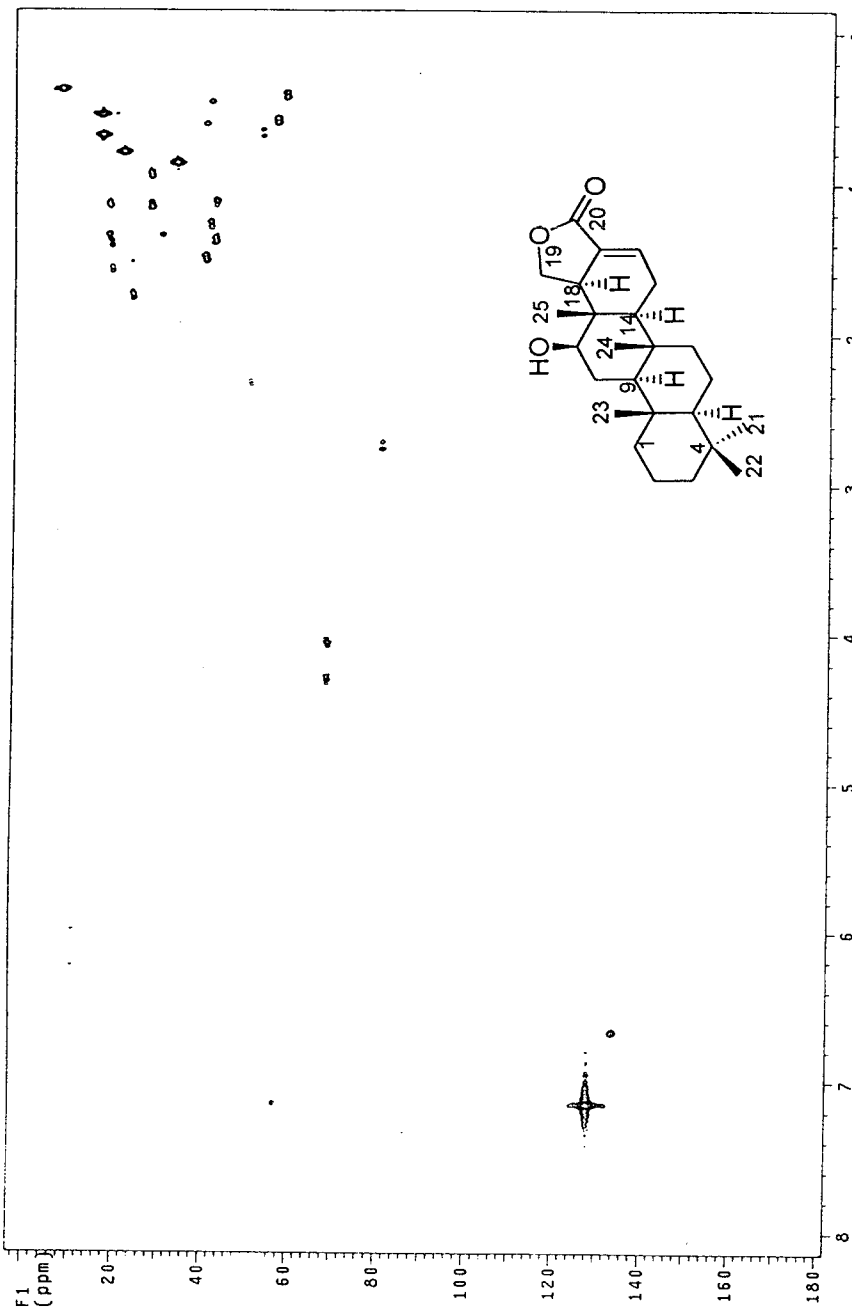

Figure 55 HMQC spectrum of **43** (500 MHz; C_6D_6)

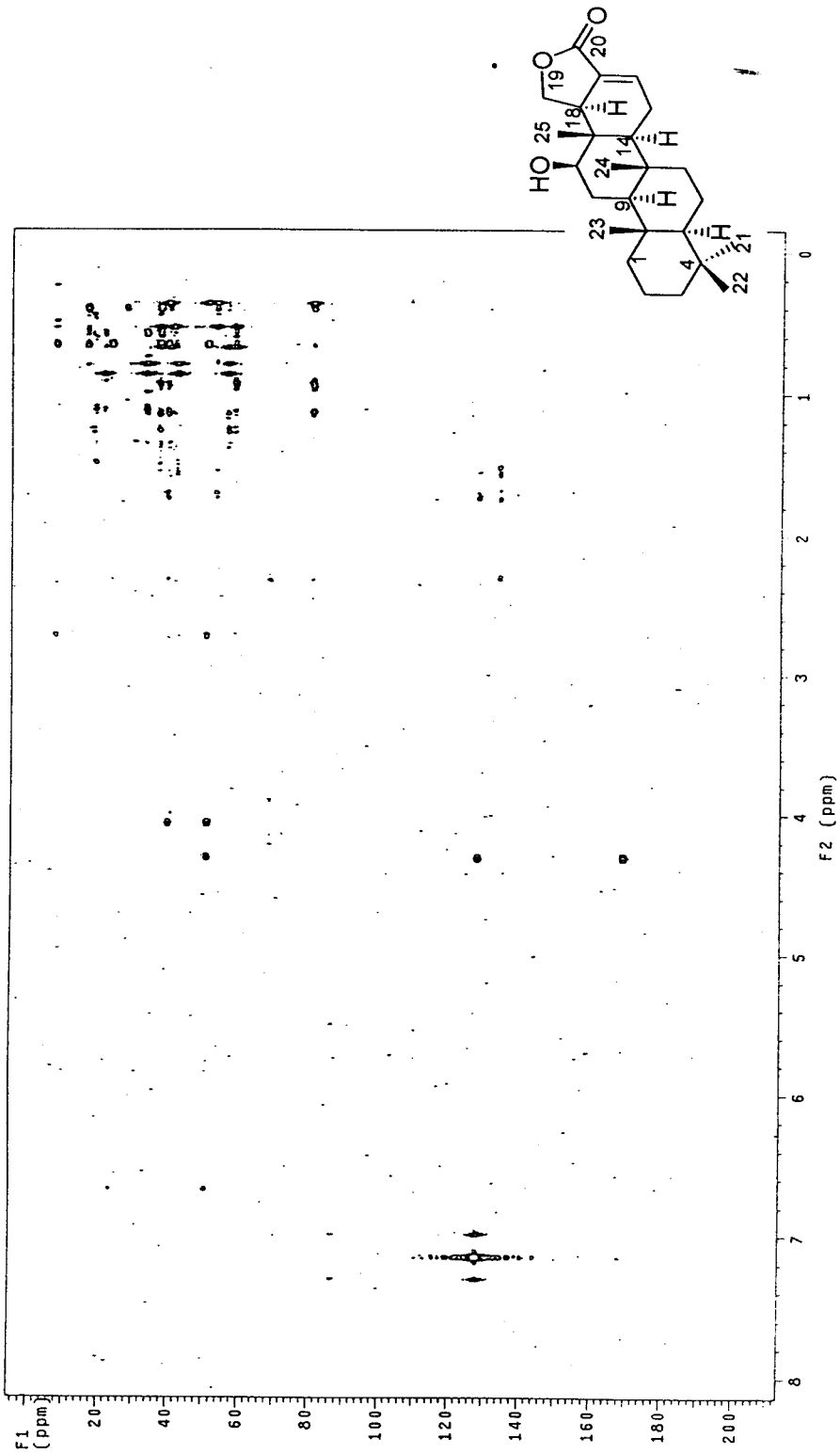

Figure 56 HMBC spectrum of **43** (500 MHz; C_6D_6)

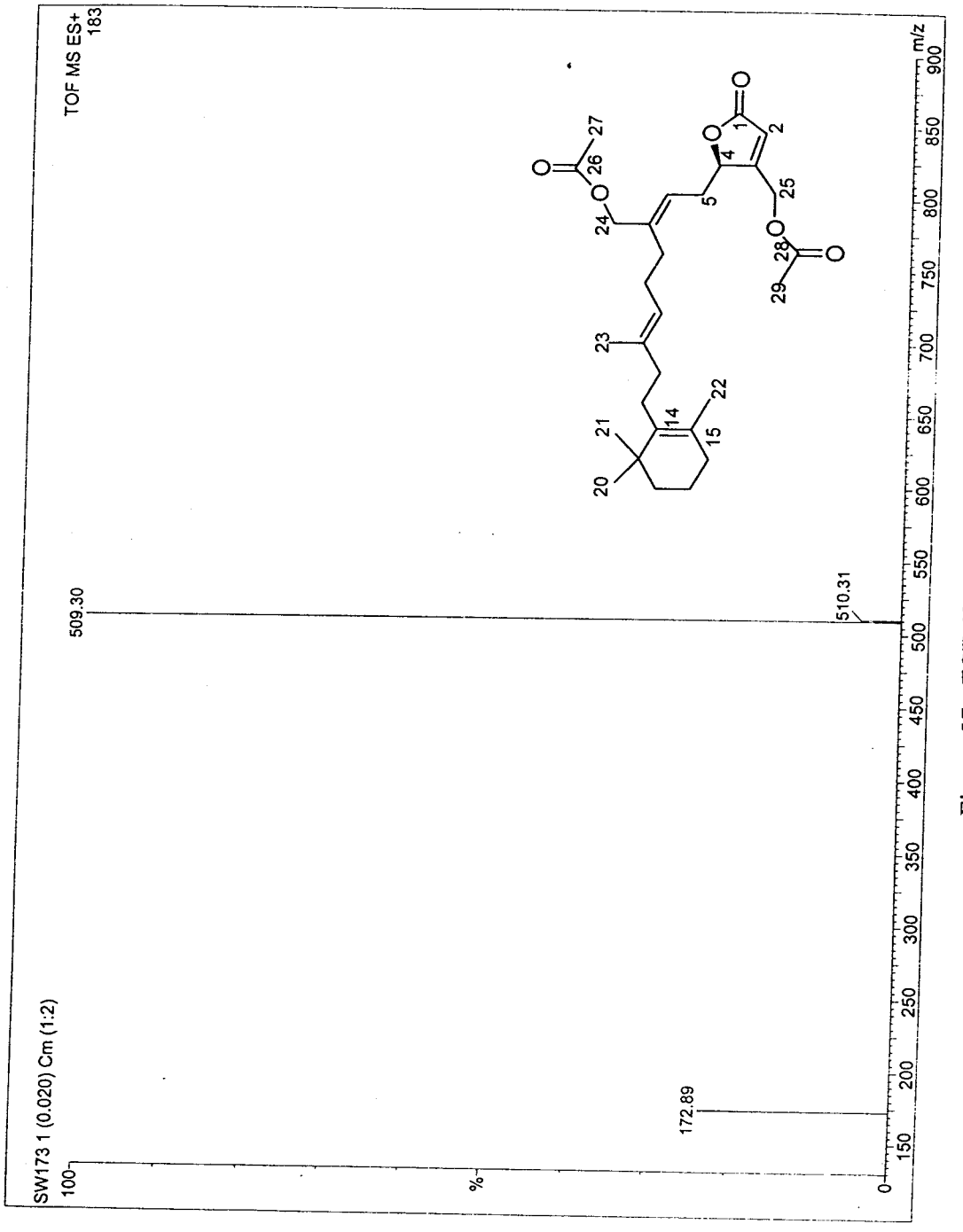

Figure 57 ESIMS spectrum of **44**

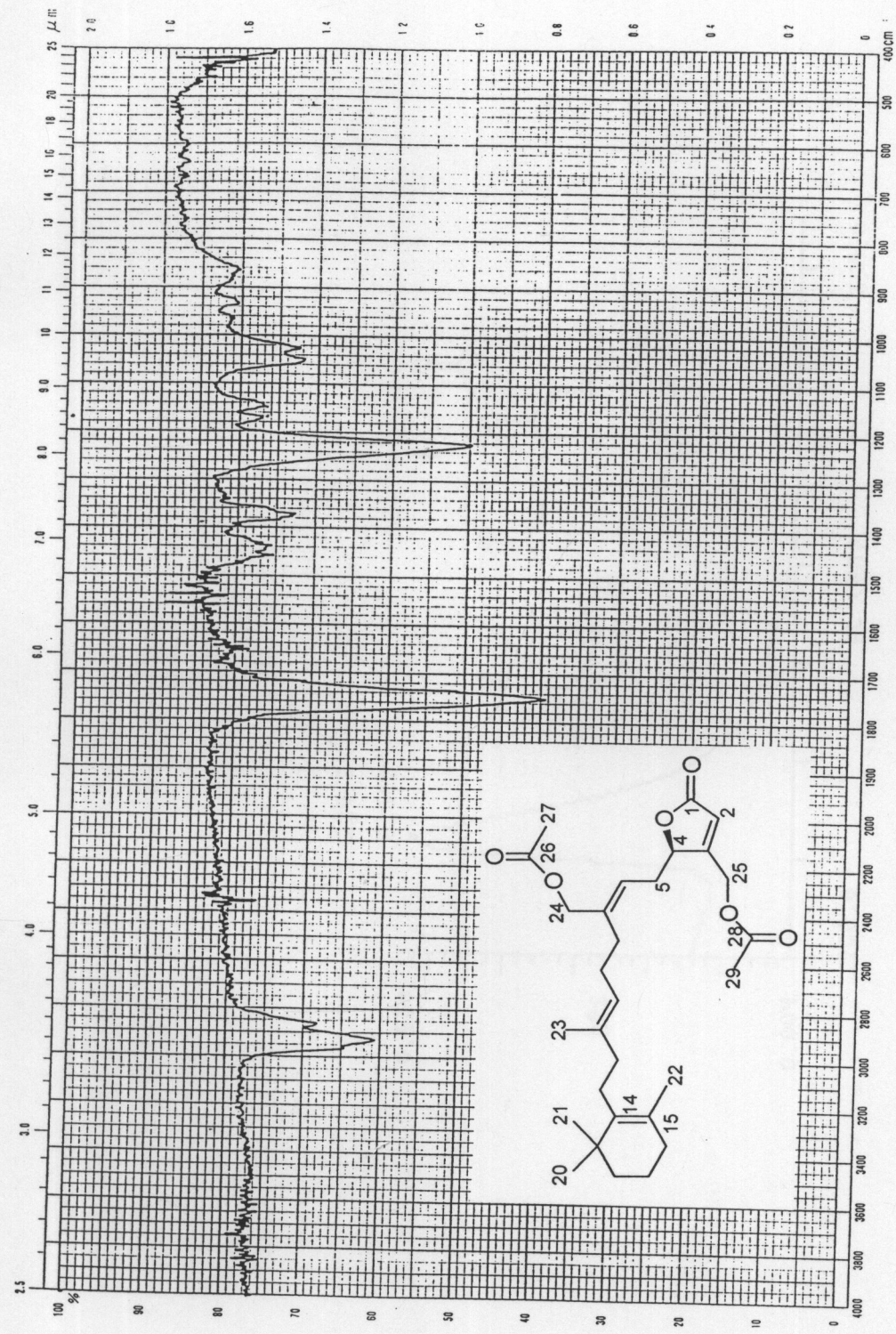

Figure 58 IR spectrum of **44** (thin film)

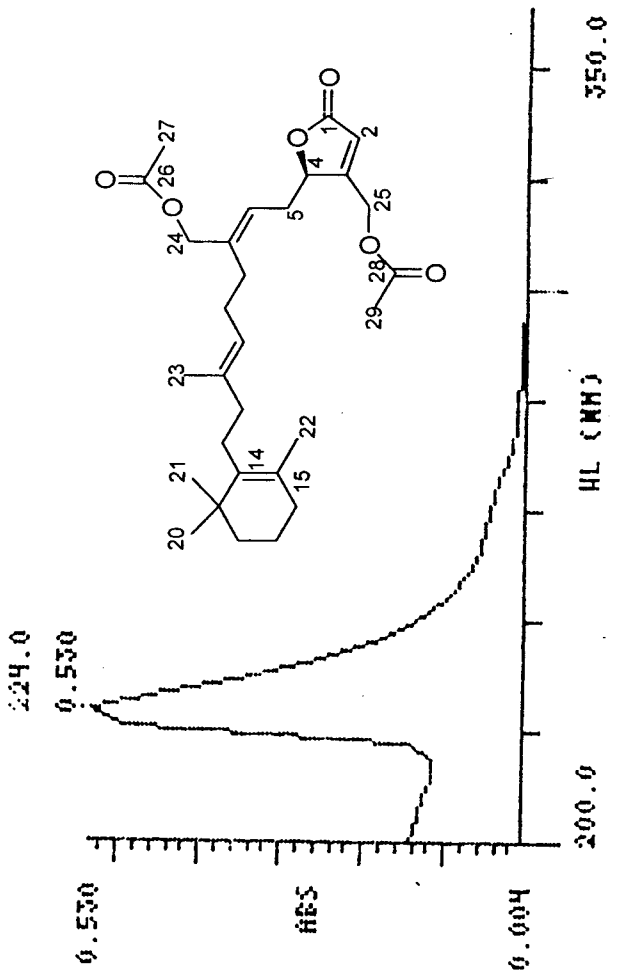

Figure 59 UV spectrum of **44** (MeOH)

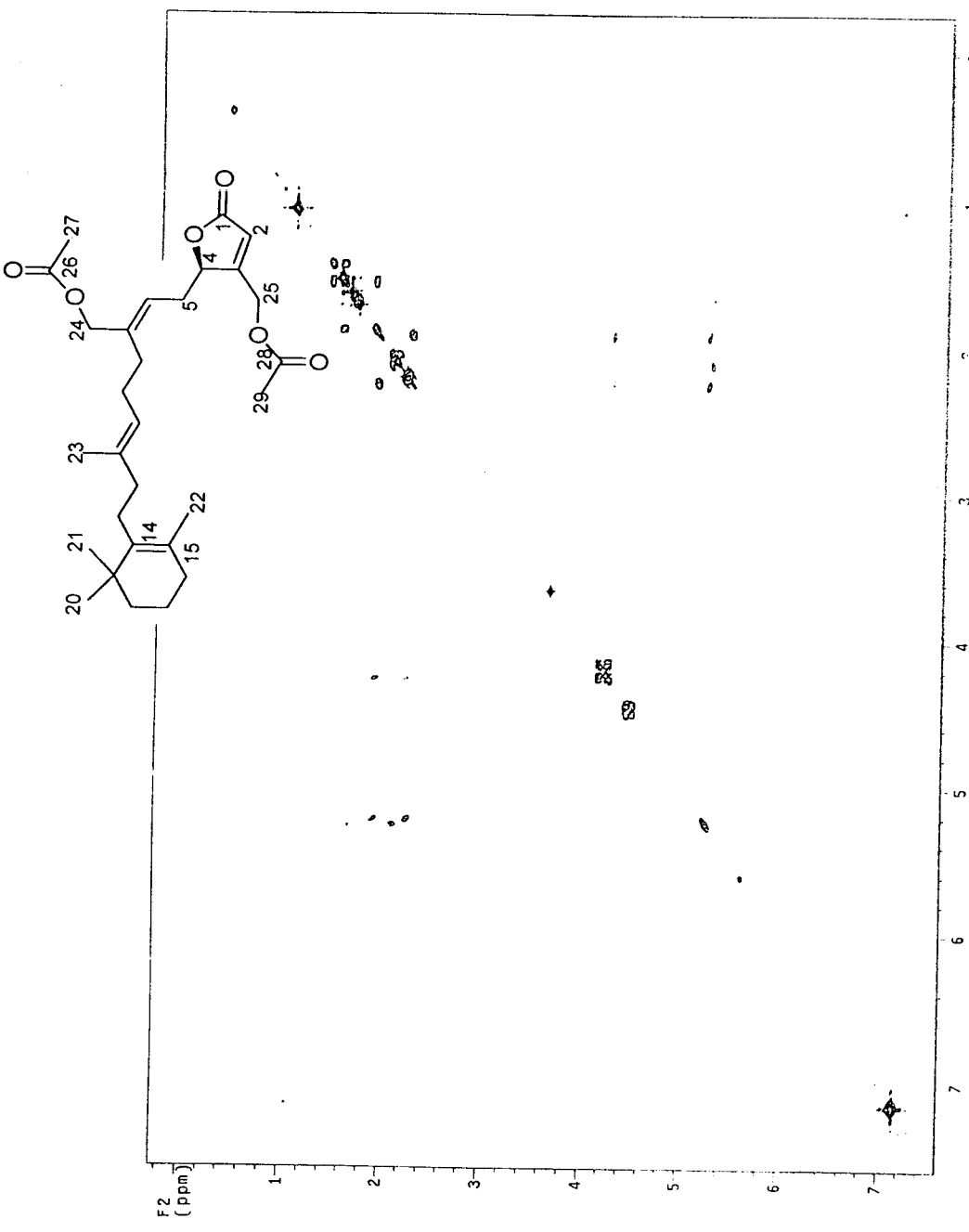

Figure 60 ^{1}H-^{1}H COSY spectrum of **44** (500 MHz; C_6D_6)

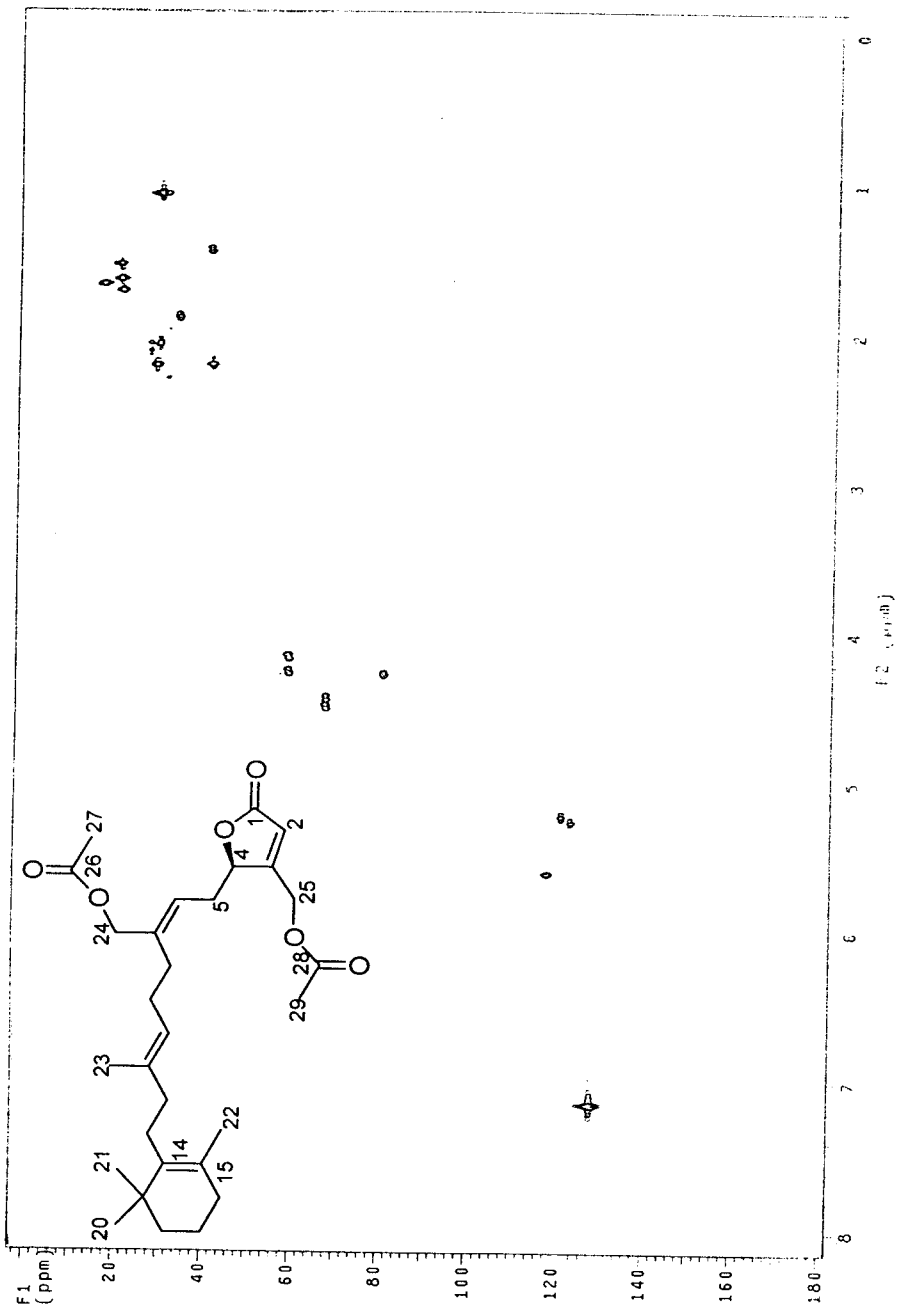

Figure 61 HMQC spectrum of **44** (500 MHz; C_6D_6)

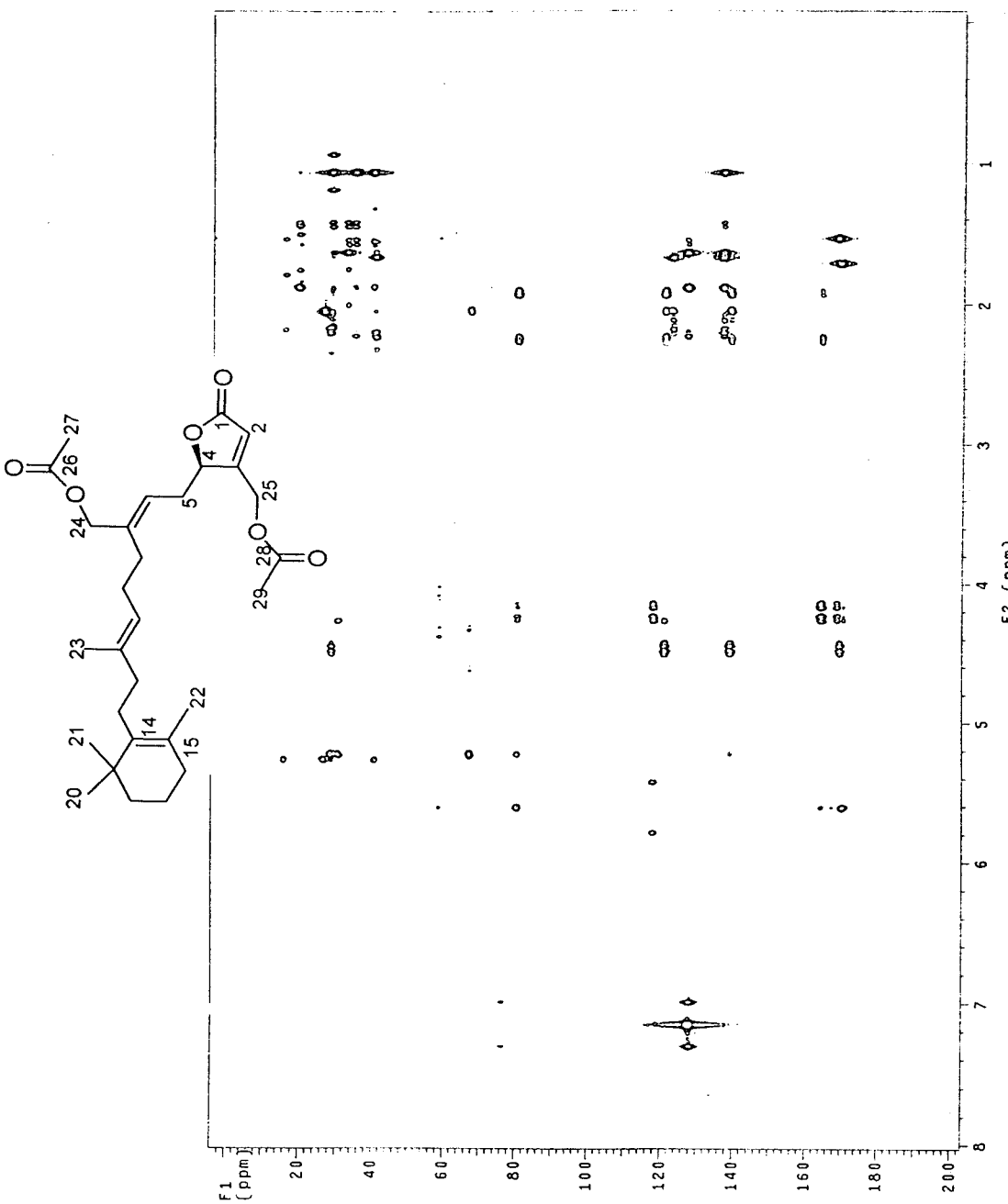

Figure 62 HMBC spectrum of **44** (500 MHz; C_6D_6)

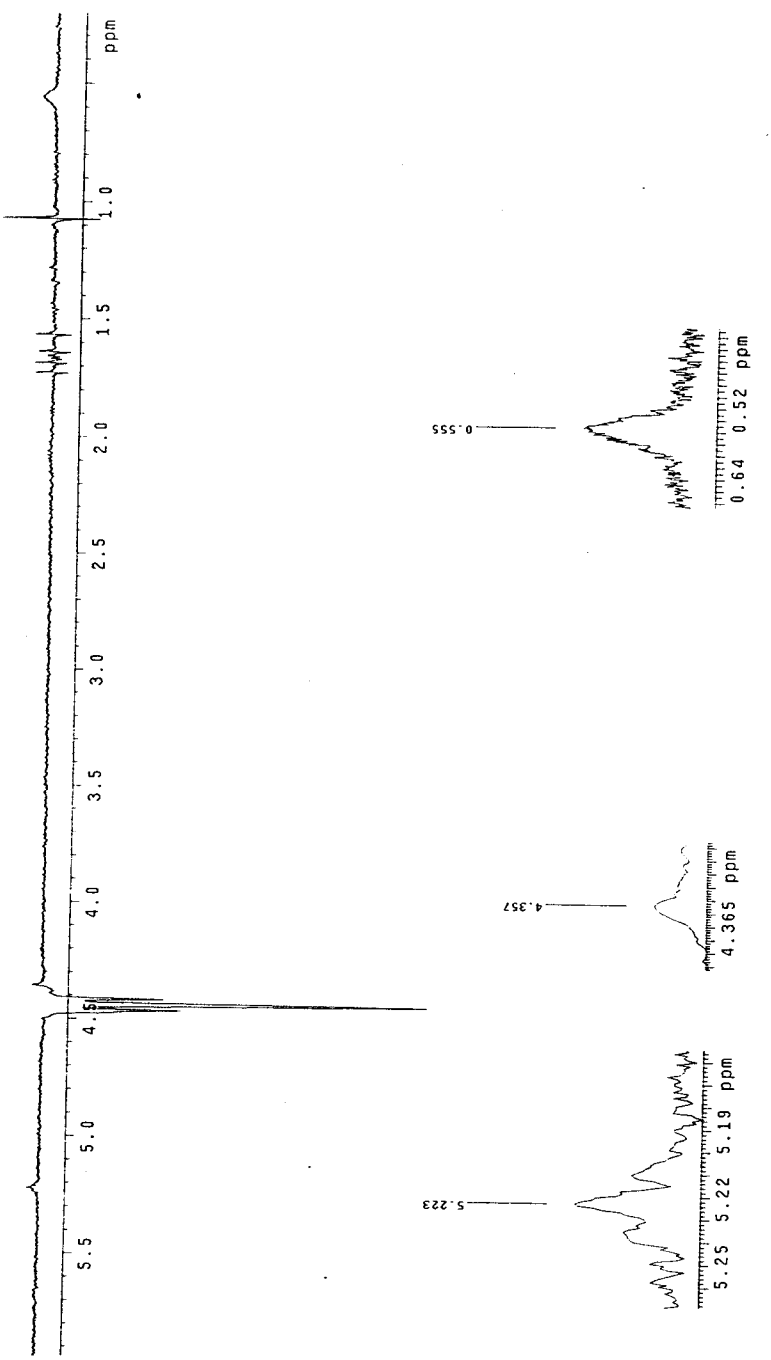

Figure 63 nOe difference spectrum of **44** after irradiation at δ_H 4.43 (H-24a)

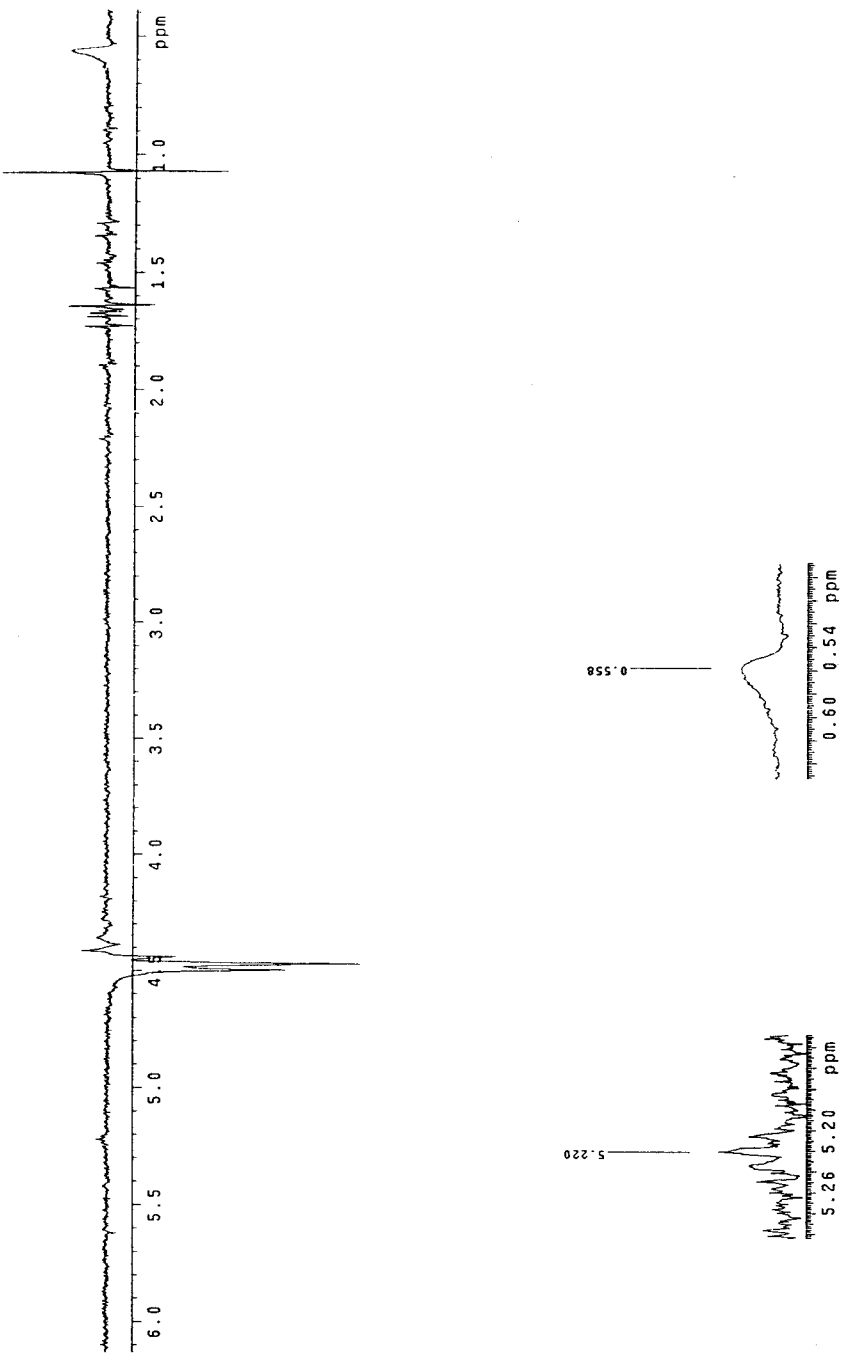

Figure 64 nOe difference spectrum of **44** after irradiation at δ_H 4.49 (H-24b)

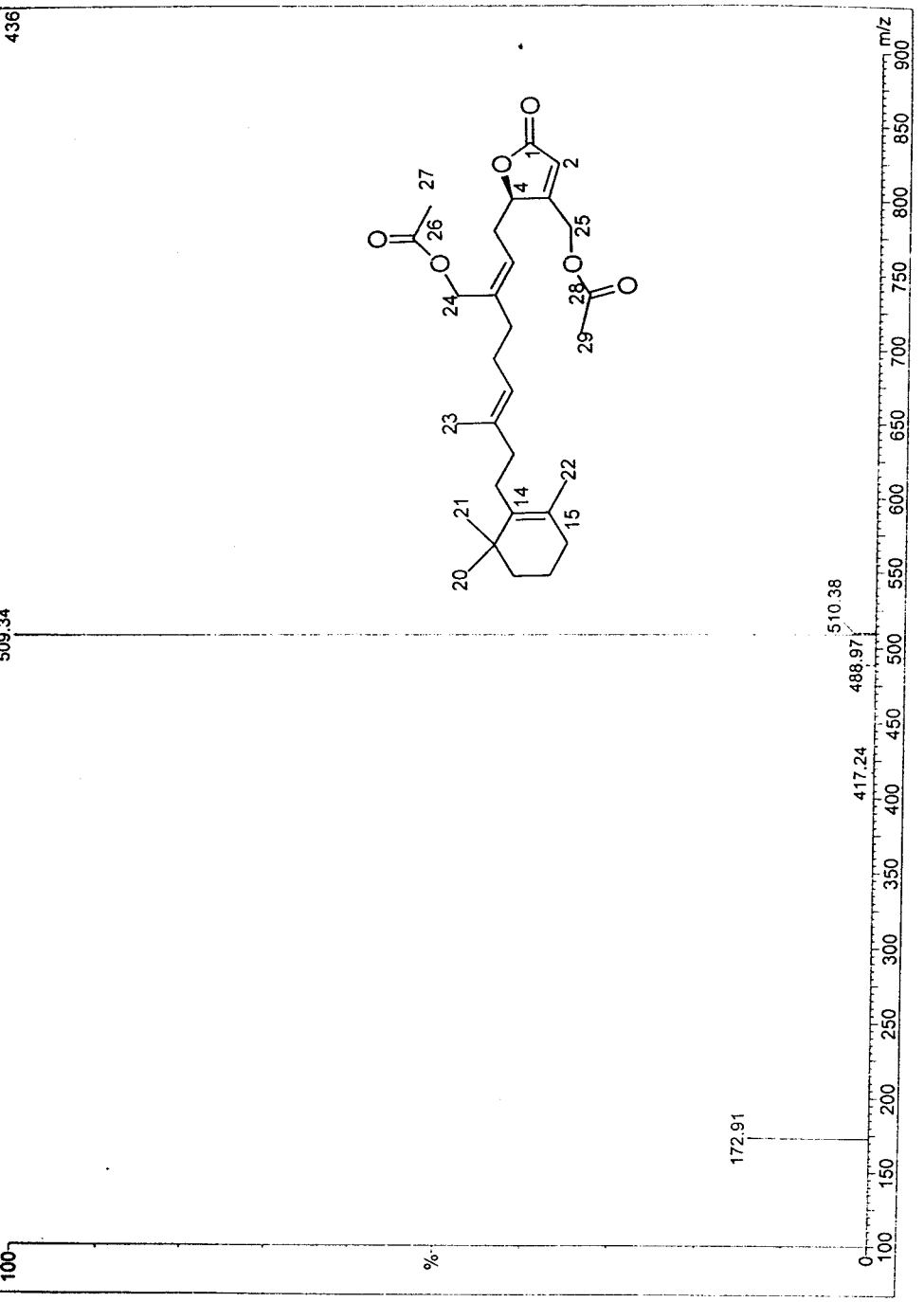

Figure 65 ESIMS spectrum of **45**

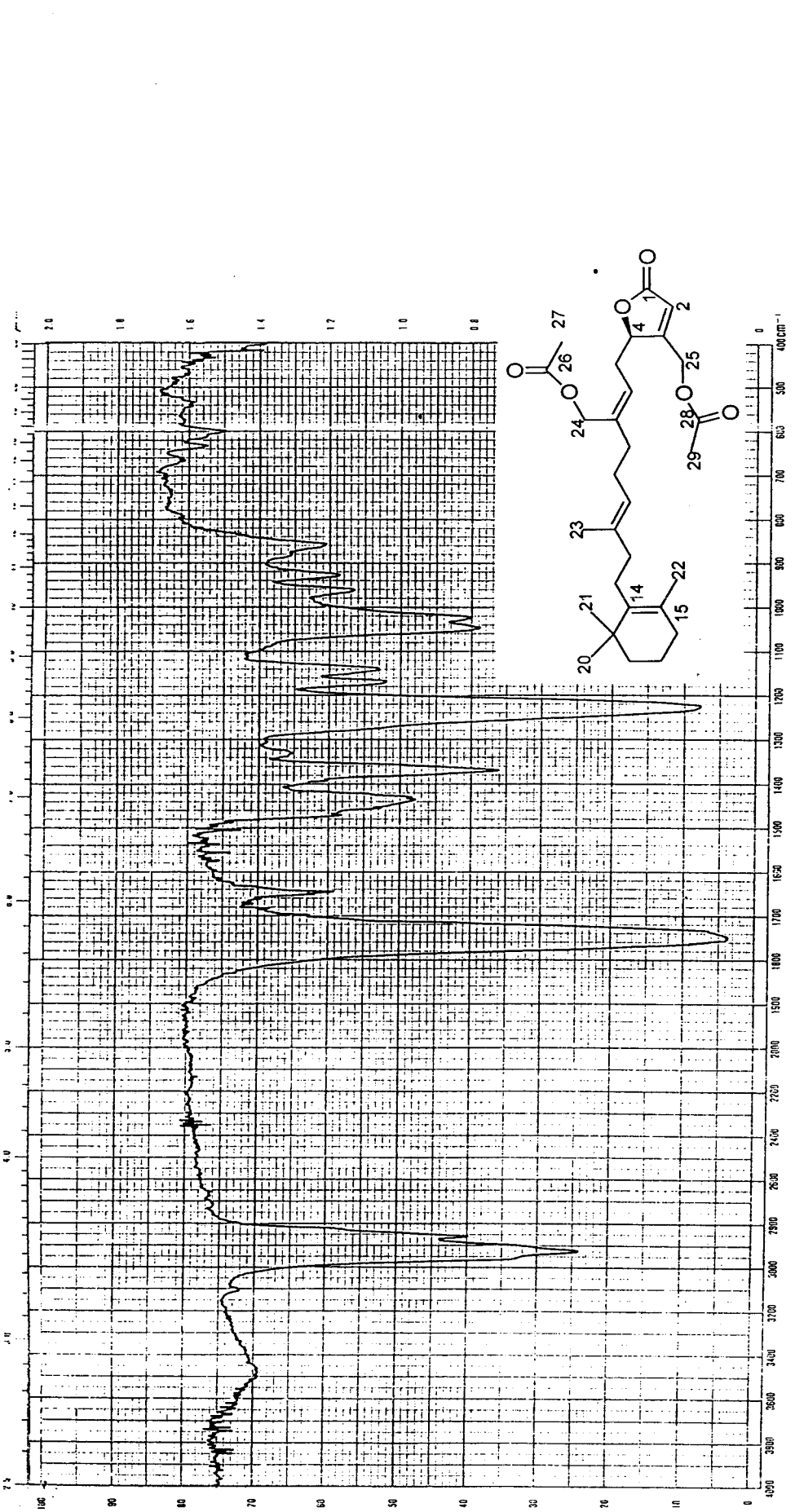

Figure 66 IR spectrum of **45** (thin film)

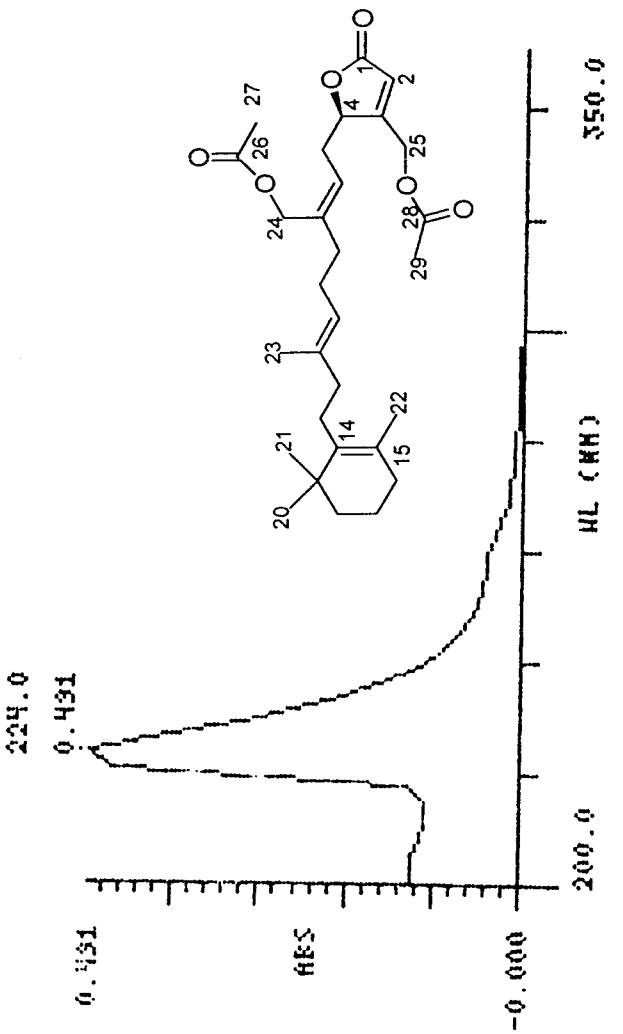

Figure 67 UV spectrum of **45** (MeOH)

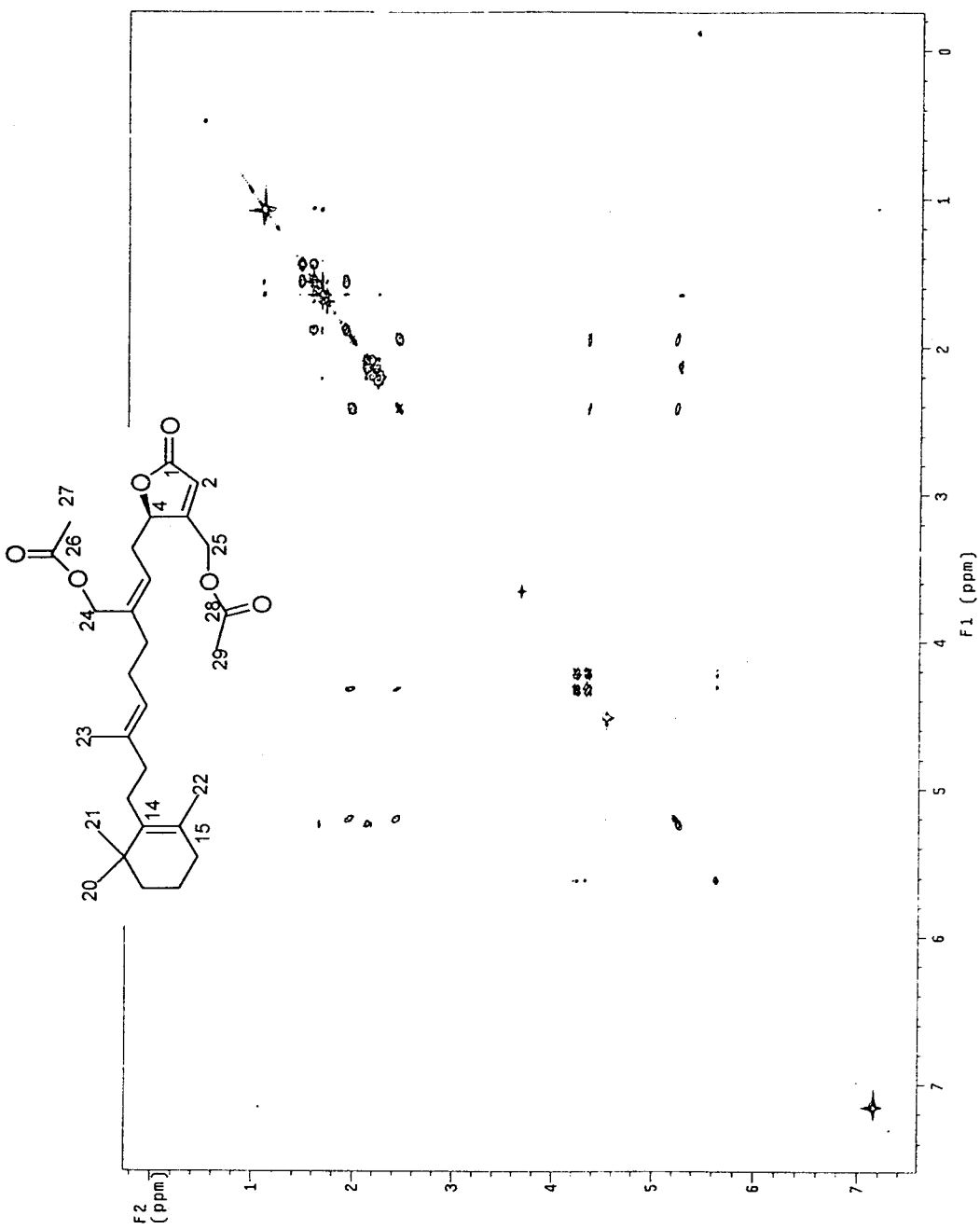

Figure 68 ^{1}H-^{1}H COSY spectrum of **45** (500 MHz; C_6D_6)

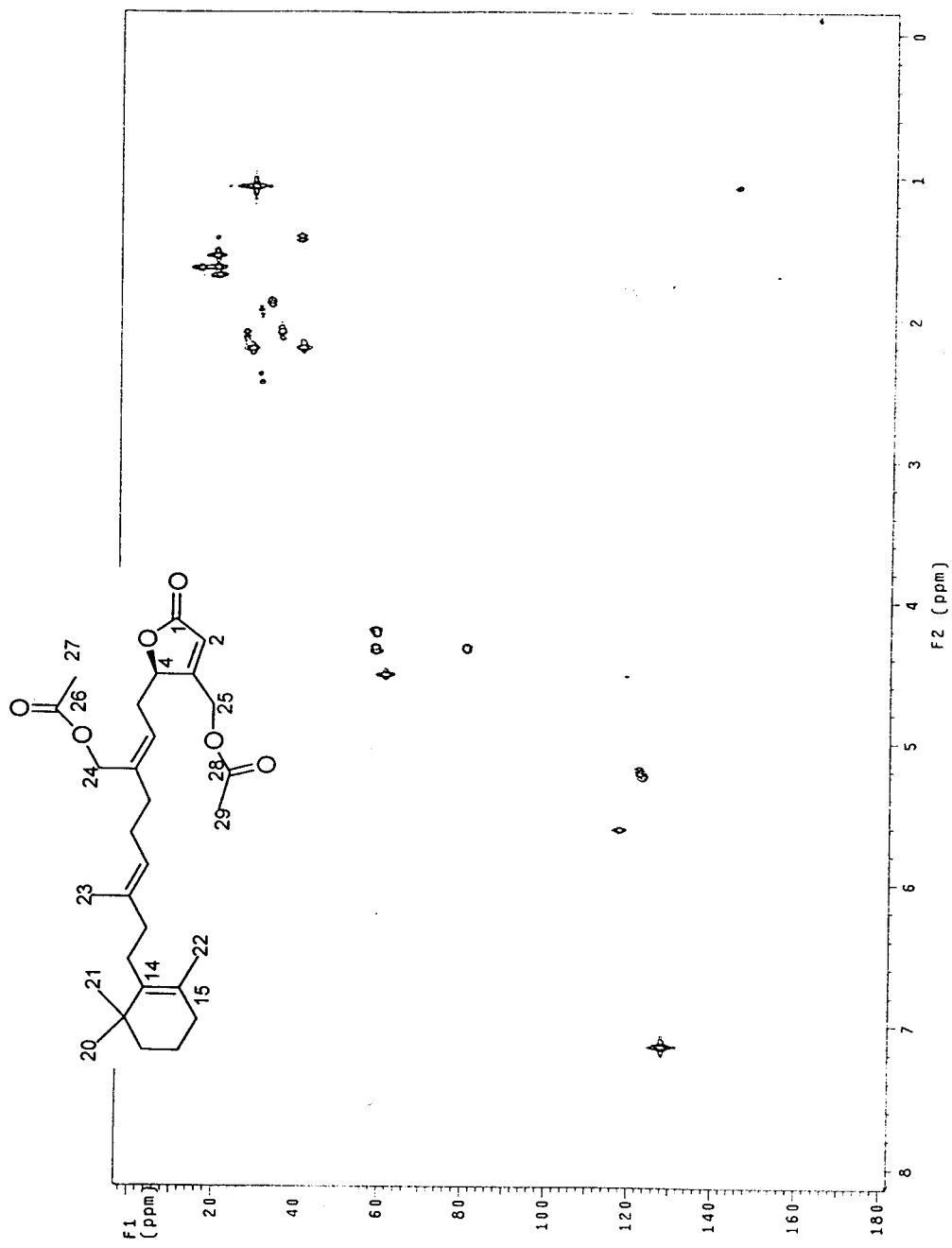

Figure 69 HMQC spectrum of **45** (500 MHz; C_6D_6)

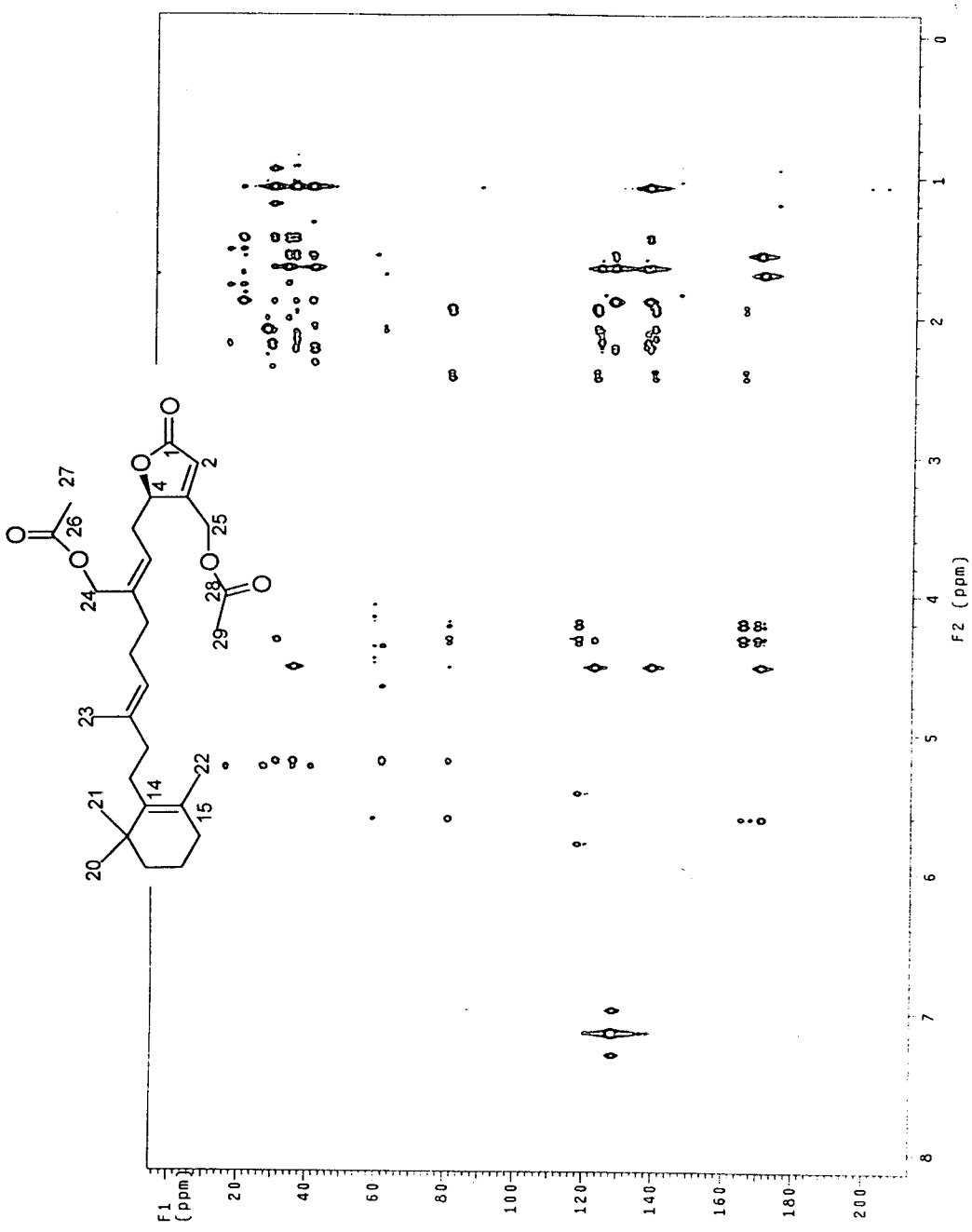

Figure 70 HMBC spectrum of **45** (500 MHz; C_6D_6)

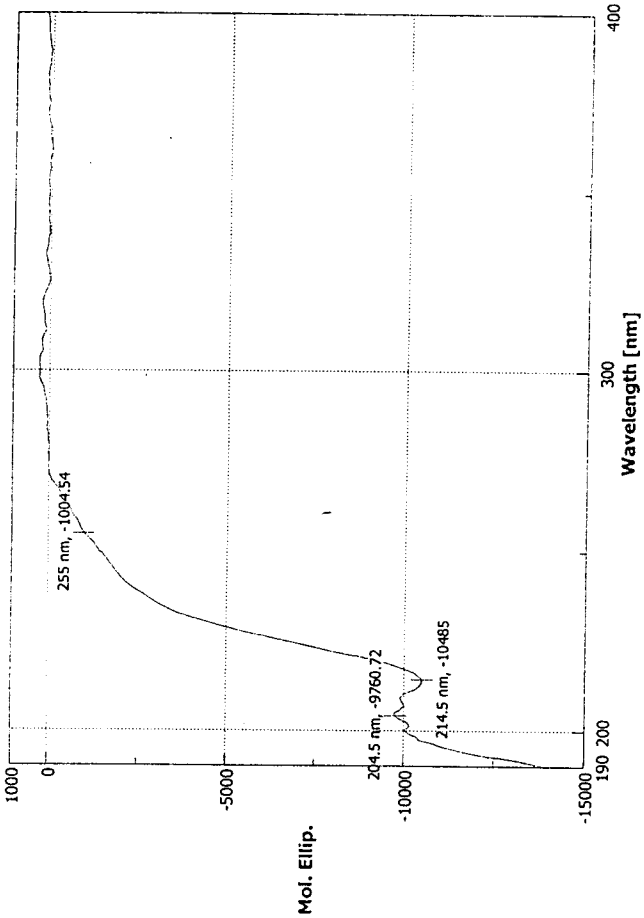

Figure 71 CD spectrum of **45** (MeOH)

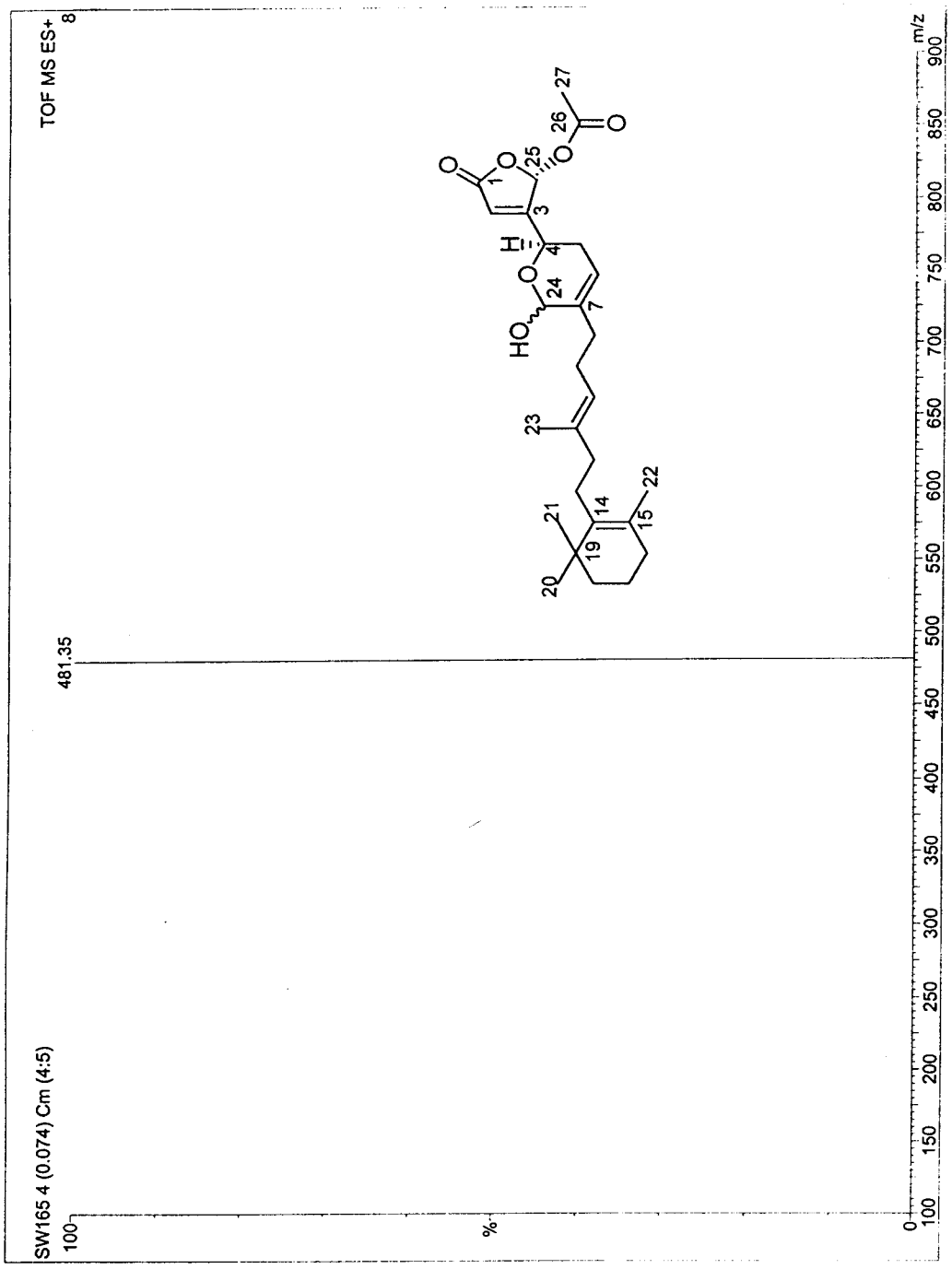

Figure 72 ESIMS spectrum of **46**

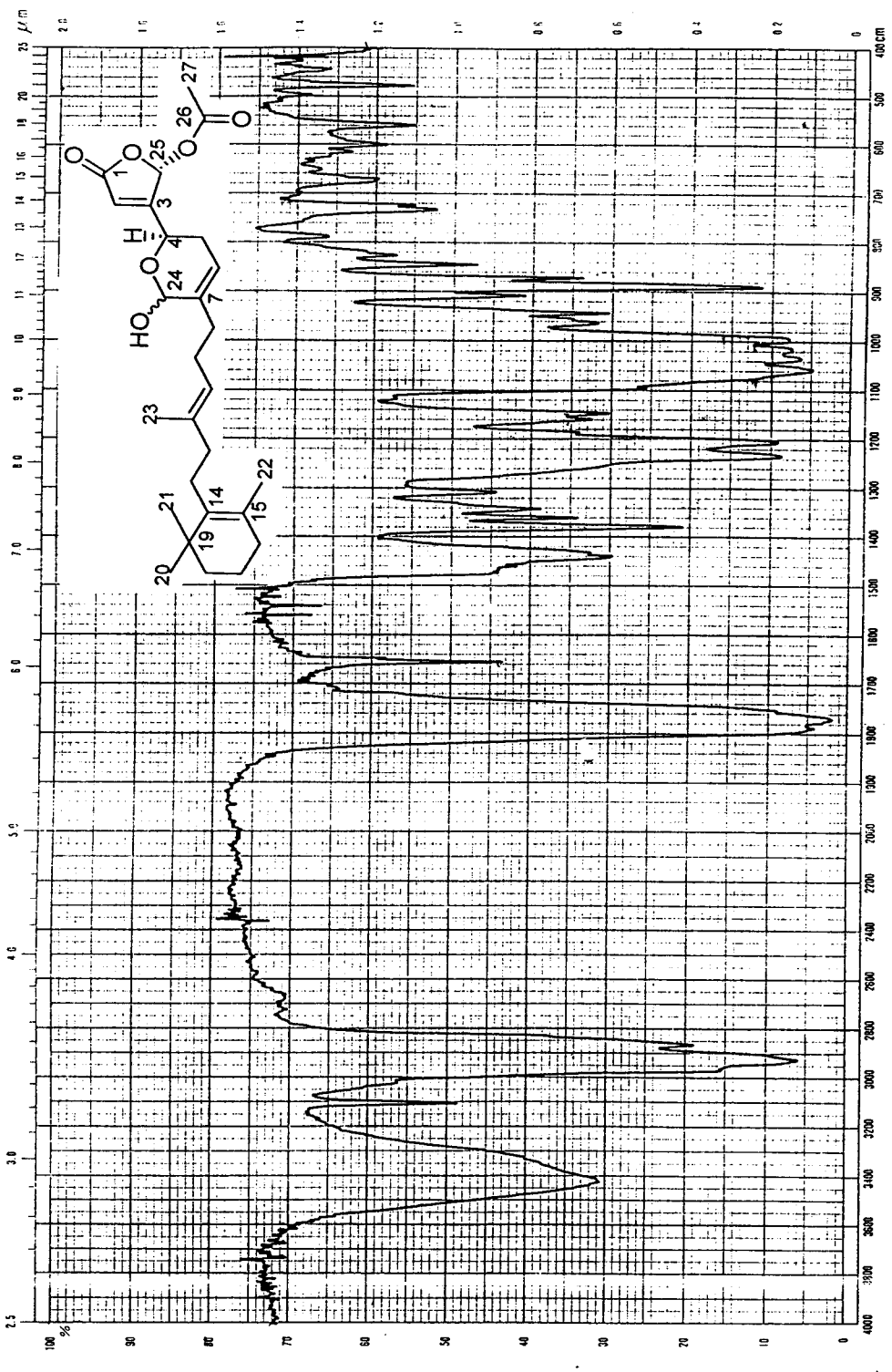

Figure 73 IR spectrum of **46** (thin film)

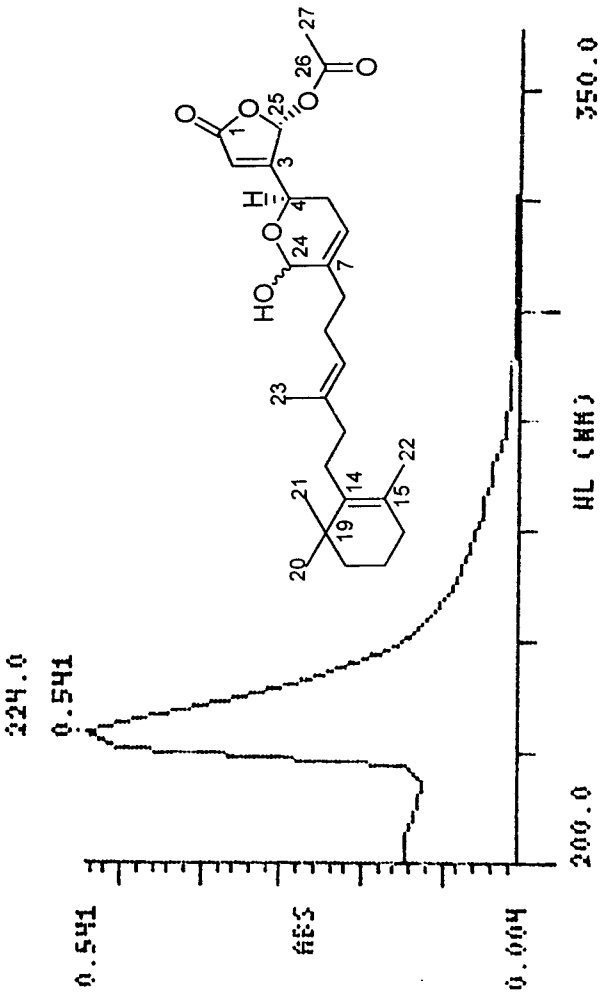

Figure 74 UV spectrum of **46** (MeOH)

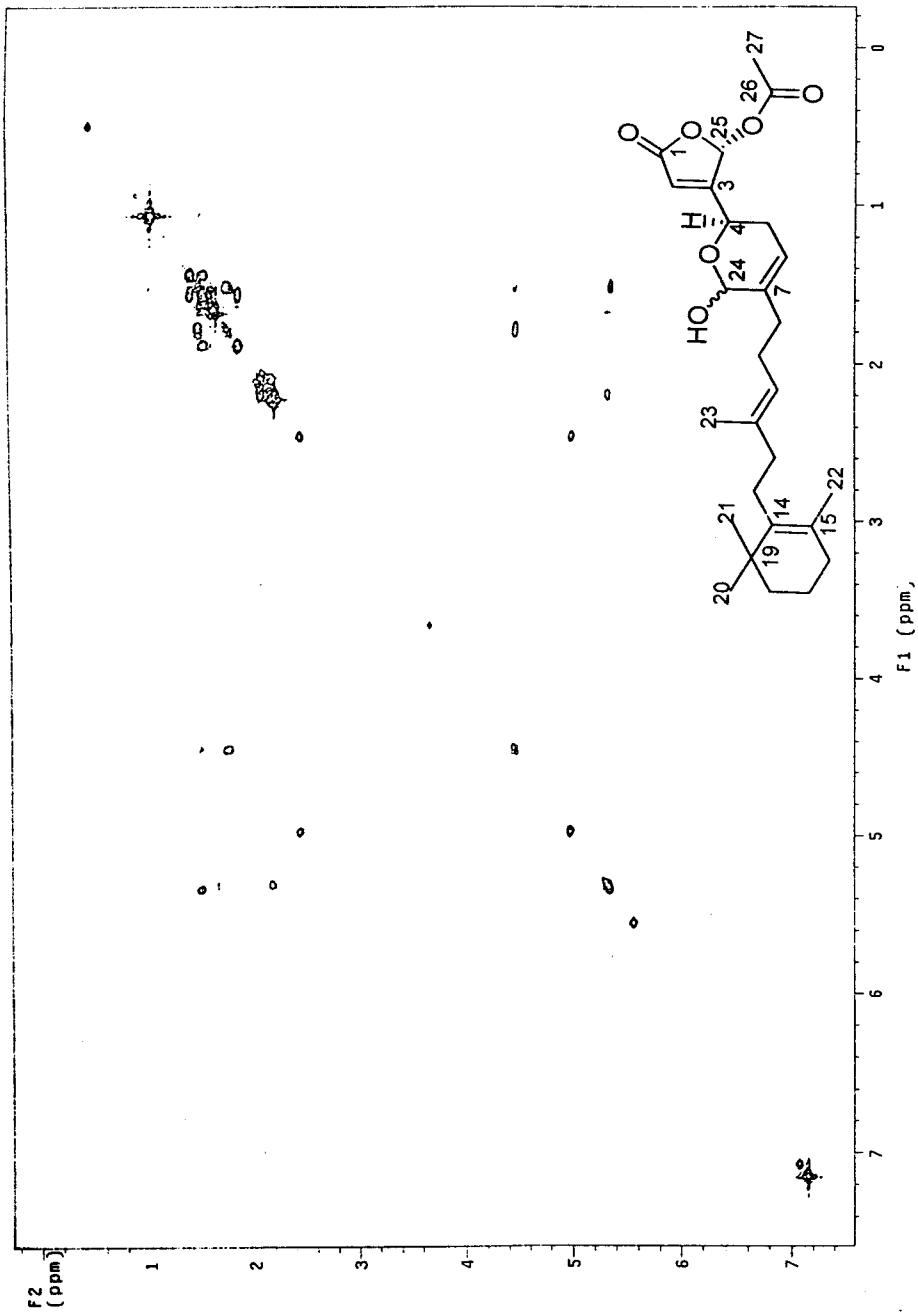

Figure 75 ^{1}H-^{1}H COSY spectrum of **46** (500 MHz; C_6D_6)

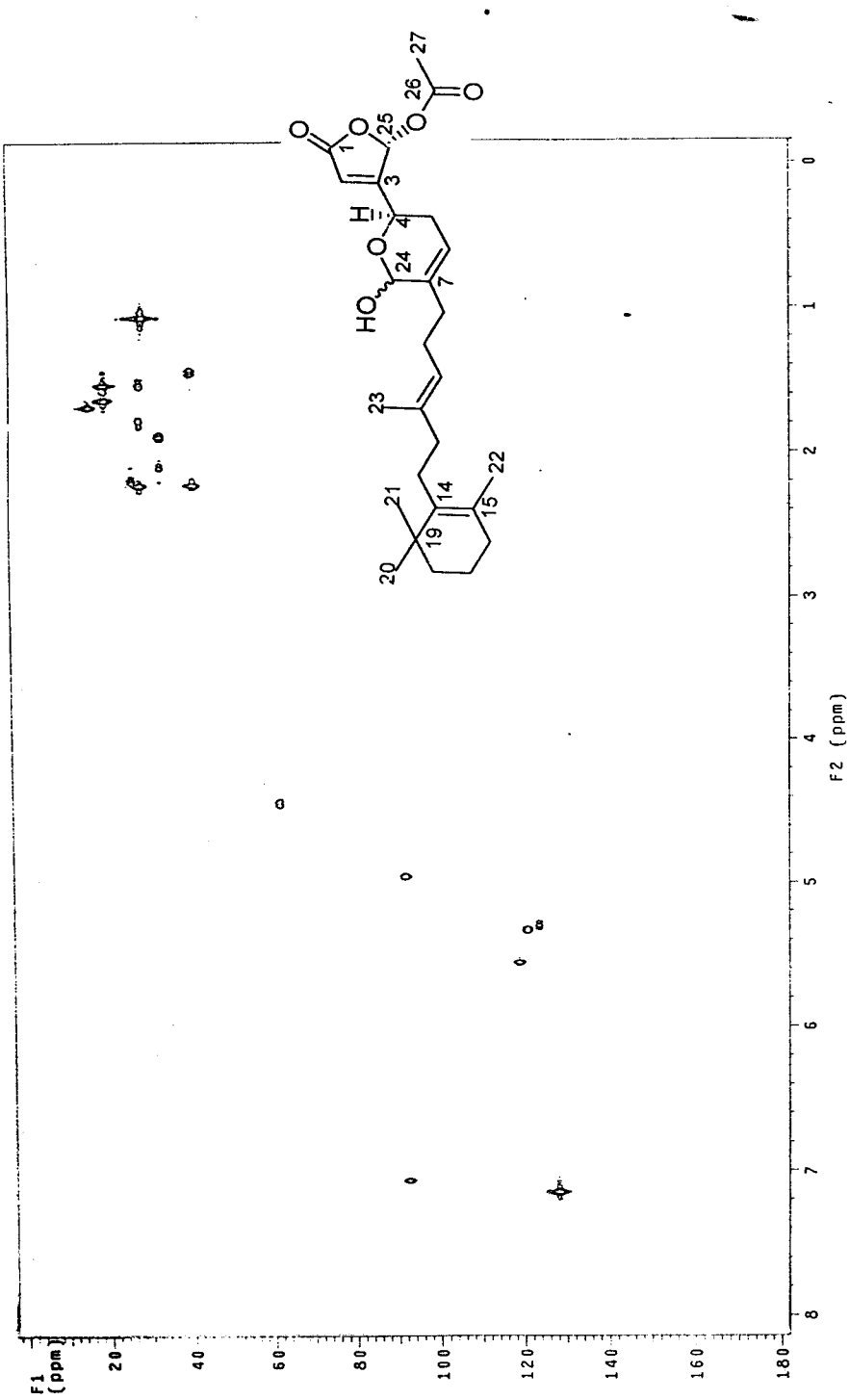

Figure 76 HMQC spectrum of **46** (500 MHz; C_6D_6)

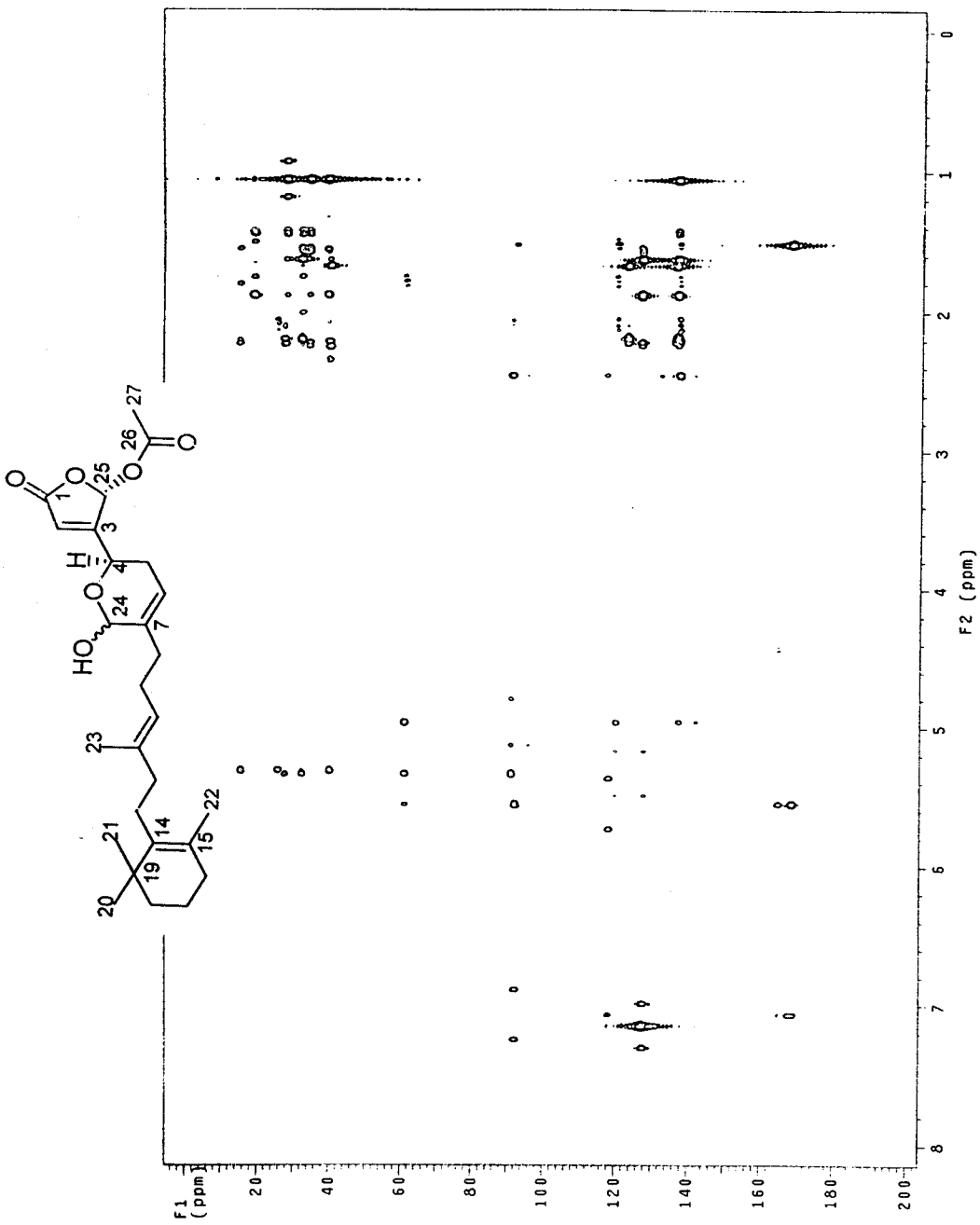

Figure 77 HMBC spectrum of **46** (500 MHz; C_6D_6)

VITAE

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