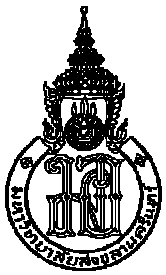

Chemical constituents from the sponges *Halichondria* sp. and *Aplysinopsis* sp.

Phatchareephorn Chatakul

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of

Master of Pharmacy in Pharmaceutical Sciences

Prince of Songkla University

2010

Thesis Title Chemical constituents from the sponges *Halichondria* sp. and *Aplysinopsis* sp.

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(Dr. Sarin Tadtong)

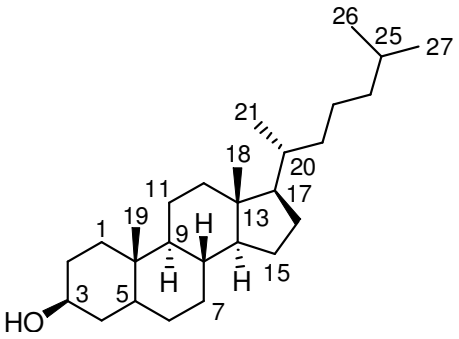
The Graduate School, Prince of Songkla University, has approved this thesis as partial fulfillment of the requirements for the Master of Pharmacy Degree in Pharmaceutical Sciences

…….
(Prof. Dr. Amornrat Phongdara)
Dean of Graduate School

ชื่อวิทยานิพนธ์ องค์ประกอบทางเคมีจากฟองน้ำสกุล *Halichondria* และฟองน้ำสกุล *Aplysinopsis*
ผู้เขียน นางสาวพัชรีพร ชาตะกูล
สาขาวิชา เภสัชศาสตร์
ปีการศึกษา 2553

บทคัดย่อ

จากการศึกษาองค์ประกอบทางเคมีจากฟองน้ำสกุล *Halichondria* และฟองน้ำสกุล *Aplysinopsis* พบว่าสารประกอบทางเคมีที่แยกสกัดได้ในชั้นเฮกเซนของฟองน้ำสกุล *Halichondria* พบสารผสมในกลุ่ม steroid ได้แก่ dihydrocholesterol (**1**) และ (3β, 24S)-stigmast-5-en-3-ol (**2**) ทำการวิเคราะห์หาสูตรโครงสร้างของสารเหล่านี้โดยเปรียบเทียบข้อมูลทางสเปกโทรสโกปีกับสารประกอบที่ทราบโครงสร้างแล้ว สำหรับการศึกษาสารประกอบทางเคมีที่แยกสกัดได้จากฟองน้ำสกุล *Aplysinopsis* สามารถแยกสารประกอบกลุ่ม sesterterpenes ได้ 5 ชนิด สารองค์ประกอบหลักทางเคมีที่แยกได้จากสารสกัดในชั้นเฮกเซน ได้แก่ heteronemin (**3**) และอีก 4 ชนิด สารที่แยกได้จากสารสกัดในชั้นไดคลอโรมีเธน ได้แก่ 12-deacetyl-12,18-di-*epi*-scalaradial (**4**), 12-deacetyl-12-*epi*-scalaradial (**5**), 12-deacetyl-12-*epi*-19-deoxyscalarin (**6**) และ 12-*epi*-scalaradial (**7**) ซึ่งสารทั้ง 5 ชนิดนี้ จัดเป็นการรายงานครั้งแรกที่พบในฟองน้ำสกุลนี้ จากการศึกษาฤทธิ์ต้านมาลาเรียในสายพันธุ์ *Plasmodium falciparum* K1 strain พบว่า **3** มีค่า IC_{50} เท่ากับ 9 μM ส่วนฤทธิ์ความเป็นพิษต่อเซลล์มะเร็งเต้านม (MCF-7) โดยใช้ sulphorhodamine B (SRB) assay พบว่า **3** มีความเป็นพิษต่อเซลล์มะเร็งสูง โดยมีค่า IC_{50} เท่ากับ 0.12 μM และฤทธิ์ต้านวัณโรคในสายพันธุ์ *Mycobacterium tuberculosis* $H_{37}Ra$ พบว่าสาร **3**, **4**, **5**, **6** และ **7** มีความแรงในระดับ MIC 6, 32, 32, 64 และ 7 μM ตามลำดับ



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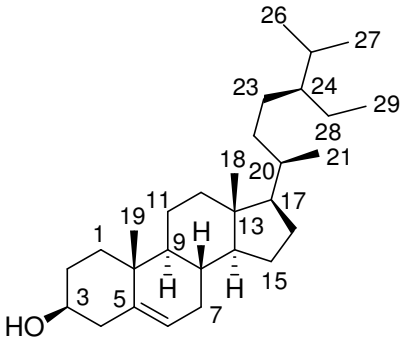

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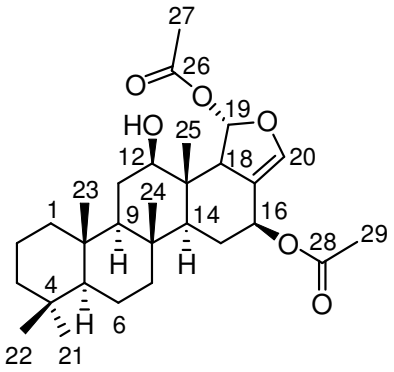

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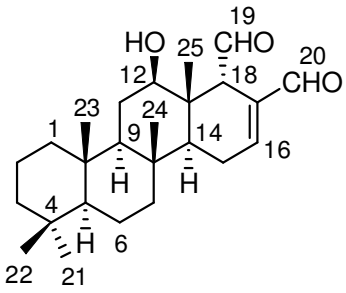

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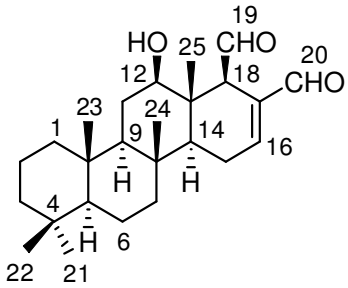

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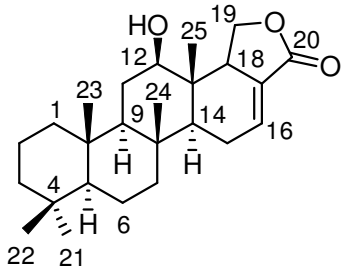

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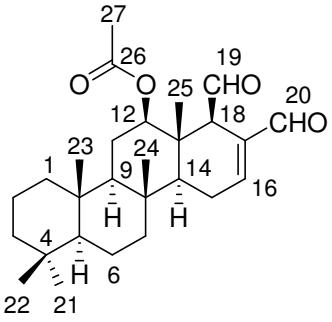

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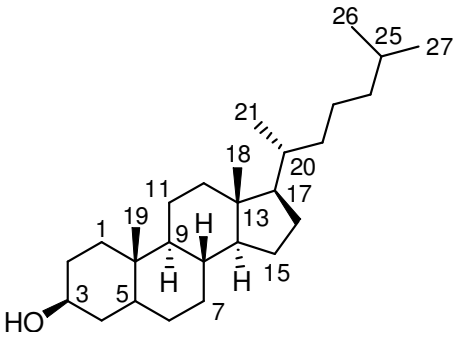
Thesis Title	Chemical constituents from the sponges *Halichondria* sp. and *Aplysinopsis* sp.
Author	Miss Phatchareephorn Chatakul
Major Program	Pharmaceutical Sciences
Academic Year	2010

ABSTRACT

Chemical constituents from the sponges *Halichondria* sp. and *Aplysinopsis* sp. The chemical investigation of the sponge *Halichondria* sp. from hexane extract, led to the isolated a mixture of two steroids dihydrocholesterol (**1**) and (3β, 24S)-stigmast-5-en-3-ol (**2**). All structures were compared of their spectroscopic data with known compounds. For the chemical investigation of the sponge *Aplysinopsis* sp. resulted in the isolation of five members of the scalarane-type sesterterpenes. Major component isolated from the hexane extract was heteronemin (**3**), and four more compounds were isolated from the dichloromethane extract including, 12-deacetyl-12,18-di-*epi*-scalaradial (**4**), 12-deacetyl-12-*epi*-scalaradial (**5**), 12-deacetyl-12-*epi*-19-deoxyscalarin (**6**) and 12-*epi*-scalaradial (**7**). All of five compounds were first reported from this sponge. The biological activity evaluation found that **3** showed antimalarial activity against *Plasmodium falciparum* K1 strain (IC_{50} 9 μM) and showed cytotoxic activity against MCF-7 cell line (IC_{50} 0.12 μM). Compounds **3**, **4**, **5**, **6**, and **7** exhibited potent antitubercular activity against *Mycobacterium tuberculosis* H_{37}Ra (MICs 6, 32, 32, 64, and 7 μM, respectively).



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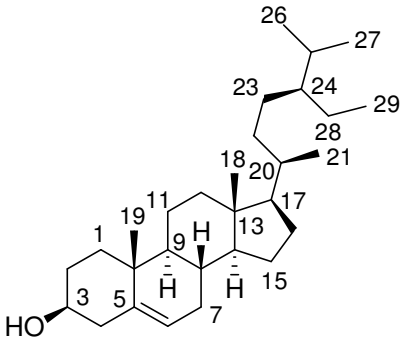

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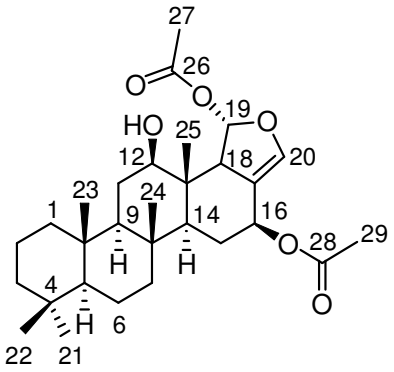

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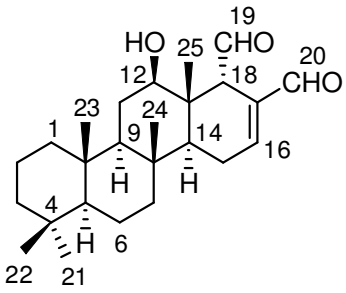

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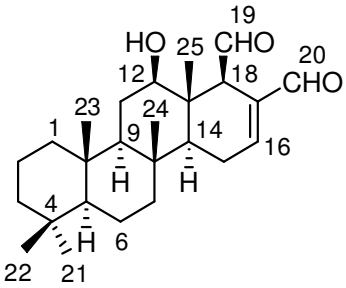

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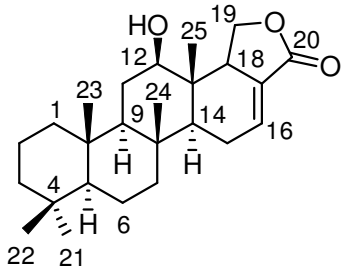

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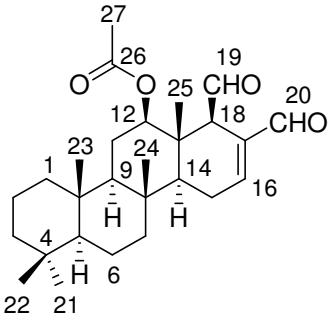

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I would like to express my grateful thank to my advisor, Assistant Professor Dr. Chatchai Wattanapiromsakul and co-advisor, Assistant Professor Dr. Anuchit Plubrukarn, for their guidance, suggestion, and assistance throughout this study.

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I would like to thank the Center of Bioactive Natural Products from Marine Organisms and Endophytic Fungi (BNPME), Faculty of Pharmaceutical Sciences, Chulalongkorn University, Graduate School, Prince of Songkla University and the Academic Excellence Enhancing Program in Pharmaceutical Sciences, Prince of Songkla University, for the financial support.

Finally, I would like to express my grateful thank to my family and all my friends for their love and encouragement.

Phatchareephorn Chatakul

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$[\alpha]_D$	specific rotation
δ	chemical shift in ppm
ε	molar extinction coefficient
λ_{max}	maximum wavelength
ν_{max}	wave number
br	broad (for NMR signals)
°C	degree Celsius
COSY	correlation spectroscopy
d	doublet (for NMR signals)
DEPT	distortionless enhancement by polarization transfer
DMSO	dimethylsulfoxide
EIMS	electron-impact mass spectroscopy
ESIMS	electrospray ionization mass spectroscopy
g	gram
GC-MS	gas chromatography-mass spectrometry
HPLC	high-performance liquid chromatography
HMBC	heteronuclear multiple bond correlation
HMQC	heteronuclear multiple-quantum coherence
Hz	hertz
IC_{50}	inhibitory concentration at 50% of tested subject
IR	infrared

LIST OF ABBREVIATIONS AND SYMBOLS (Continued)

J	coupling constant
$[M]^+$	molecular ion
MHA	mueller hinton agar
MIC	minimum inhibitory concentration
m	multiplet (for NMR signals)
mg	milligram
min	minute
mL	milliliter
m/z	mass-over-charge ratio
μg	microgram
μL	microliter
μM	micromolar
MS	mass spectrometry
nOe	nuclear Overhauser effect
NMR	nuclear magnetic resonance
q	quartet (for NMR signals)
ppm	part per million
s	singlet (for NMR signals)
SDA	sabouraud dextrose agar
SRB	sulphorhodamine B
t	triplet (for NMR signals)

LIST OF ABBREVIATIONS AND SYMBOLS (Continued)

TB	tuberculosis
TLC	thin layer chromatography
UV	ultraviolet

CHAPTER 1

INTRODUCTION

1.1 General introduction

Today, the studies of marine natural products are interesting for researchers around the world. Marine organisms have the potential to be sources of new compounds equal to terrestrial plants and other organisms. The chemicals from marine natural products are derived and currently used in clinic such as cytarabine and gemcitabine. These compounds are arabinonucleosides derivatives that used in chemotherapy agent. The first drug from the sea was ziconotide which valuable in commercial under trade name “Prialt” which is potent peptide toxins from cone snails (Simmons *et al*., 2005). Other compounds are currently being investigated in clinical trials for the drug such as ecteinascidin 743 and aplidine. The compounds used as chemotherapeutic agents (Molinski *et al*., 2009).

Sponges are sources of secondary metabolites (Blunt *et al*., 2005; Faulkner, 2002). The bioactive compounds of sponge are very diverse in both structure and bioactivity. There are several types of compounds such as polyketides, alkaloids, sterols, cyclic peptides, and terpenes. Most compounds exhibit interesting bioactivities, including antiviral, anticancer, antiparasitic, anti-inflammatory, antimalarial, and antibiotic activities as well as herbicidal and antifouling properties (Osinga *et al*., 1998).

1.2 Chemical constituents from the sponge of the genus *Halichondria*

Many groups of compounds have been isolated from the sponge genus *Halichondria* such as terpenoids, polyethers/macrolides, alkaloids and miscellaneous compounds.

1.2.1 Terpenoids (Table 1)

Terpenoids consist of isoprene units (C-5 unit) in their carbon skeletons. Terpenes which isolated from *Halichondria* sp. are sesquiterpenes, diterpenes, and sesterterpenes.

1.2.1.1 Sesquiterpenes

Sesquiterpenes consist of three isoprene units and having fifteen carbon atoms (C15-compounds). Three aromatic sesquiterpenes were isolated from *Halichondria* sp., (1′*Z*)-2-(1′,5′-dimethylhexa-1′,4′-dienyl)-5-methylbenzene-1,4-diol (**1**), (1′*E*)-2-(1′,5′-dimethylhexa-1′,4′-dienyl)-5-methylbenzene-1,4-diol (**2**), and (1′*E*)-2-(1′,5′-dimethylhexa-1′,4′-dienyl)-5-methylphenyl acetate (**3**) (Capon *et al*., 1982).

Nitrogenous sesquiterpenes, 3-isocyanobisabolane-8,10-diene (**4**), 3-formamidobisabolane-8,10-diene (**5**), 3-isocyanotheonellin (**6**), and 3-formamidotheonellin (**7**) were isolated from *Halichondria* cf. *lendenfeldi* (Kassuhlke *et al*., 1991). (6*R*,7*S*)-7-Amino-7,8-dihydro-α-bisabolene (**8**) was isolated from *Halichondria* sp. (Sullivan *et al*., 1986). 10-Isothiocyanato-4-amorphene (**9**) was isolated from *Halichondria* sp. (Garson and Simpson, 2004; Erpenbeck *et al*., 2005; Faulkner *et al*., 2004). Halipanicine (3-isothiocyanato-1(10)-cadinene) (**10**) was isolated from *Halichondria panicca* (Nakamura *et al*., 1991). Halichonadins B-D (**11-**

13) (Ishiyama *et al.,* 2005) and halichonadin F (**14**) were isolated from *Halichondria* sp. (Ishiyama *et al.,* 2008) and nitrogenous sesquiterpenes derivertive, halichonadin A (**15**) (Ishiyama *et al.,* 2005) and halichonadin E (**16**) were isolated from *Halichondria* sp. (Kozawa *et al*., 2008).

1.2.1.2 Diterpenes

Diterpenes consist of four isoprene units and having twenty carbon atoms (C20-compounds). Six nitrogenous diterpenes were isolated from *Halichondria* sp., 7-isocyano-1-cycloamphilectene (**17**), 7-isocyano-11-cycloamphilectene (**18**), 8-isocyano-l0,14-amphilectadiene (**19**), 8-isocyano-1(12)-cycloamphilectene (**20**) (Molinski *et al*., 1987), and formamido-substituted cycloamphilectenes (**21** and **22**) (Garson and Simpson, 2004).

1.2.1.3 Sesterterpenes

Sesterterpenes consist of five isoprene units and having twenty five carbon atoms (C25-compounds). Homosesterterpene (**23**) was isolated from *Halichondria* sp. (Nakagawa *et al*., 1987) and steroid, hilistanol sulfate (**24**) was isolated from *Halichondria* cf. *moorei* Bergquist (Fusetani *et al.,* 1981).

1.2.2 Polyethers/macrolides (Table 2)

Polyether macrolides, halichondrin B (**25**), C (**26**), homohalichondrin A-C (**27-29**) (Hirata and Uemura, 1986), norhalichondrin A (**30**) (Uemura *et al*., 1985), norhalichondrin B (**31**) and C (**32**) were isolated from *Halichondria okadai* Kadota (Hirata and Uemura, 1986).

Macrolides, halichondramide (**33**) (Kernan and Faulkner, 1987), dihydrohalichondramide (**34**), isohalichondramide (**35**) (Kernan *et al*., 1988), halishigamide A (**36**) and B (**37**) were isolated from *Halichondria* sp. (Kobayashi *et al*., 1997) and okadaic acid (**38**) was isolated from *Halichondria okadai* Kadota and *H. melonodocia* (Tachibana *et al*., 1981).

1.2.3 Alkaloids (Table 3)

Two isoquinoline quinine alkaloids were isolated from *Halichondria* sp., mimosamycin (**39**) and *O*-demethylrenierone (**40**) (Sung *et al*., 2006). Tetracyclic alkylbipiperidine alkaloid, halichondramine (**41**) was isolated from *Halichondria* sp. (Chill *et al*., 2002) and cyclic polyketide alkaloid, halichlorine (**42**) was isolated from *Halichondria okadai* Kadota (Kuramoto *et al*., 1996).

1.2.4 Miscellaneous compounds (Table 4)

Halishigamide C (**43**) and D (**44**) were isolated from *Halichondria* sp. (Kobayashi *et al*., 1997). Glycoshingolipids, halicylindrosides A_1-A_4 (**45-48**) and B_5-B_{10} **49-54**) were isolated from *Halichondria cylindrata* and *H. panacea* (Li *et al*., 1995). Tetramic acid lactam, cylindramide (**55**) was isolated from *Halichondria cylindrata* (Kanazawa *et al*., 1993). Five tetradecapeptides were isolated from *Halichondria cylindrata*, halicylindramides A-C (**56-58**) (Li *et al*., 1995), halicylindramide D (**59**) and E (**60**) (Li *et al*., 1996). Fatty acid derivertives, halichonlactone (**61**) and neohalichonlactone (**62**) were isolated from *Halichondria okadai* Kadota (Niwa *et al*., 1989).

Table 1 Terpenoids from the sponges of genus *Halichondria*

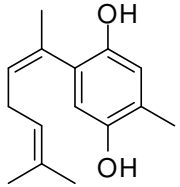
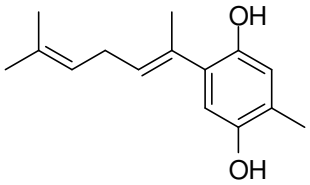
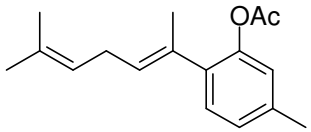
Names/ Structures	Sources	Activities	References
aromatic sesquiterpenes			
(1′*Z*)-2-(1′,5′-dimethylhexa-1′,4′-dienyl)-5-methylbenzene-1,4-diol (**1**) 	*Halichondria* sp.	N/A	(Capon *et al*., 1982)
(1′*E*)-2-(1′,5′-dimethylhexa-1′,4′-dienyl)-5-methylbenzene-1,4-diol (**2**) 	*Halichondria* sp.	N/A	(Capon *et al*., 1982)
(1′*E*)-2-(1′,5′-dimethylhexa-1′,4′-dienyl)-5-methylphenyl acetate (**3**) 	*Halichondria* sp.	N/A	(Capon *et al*., 1982)

Table 1 (cont.)

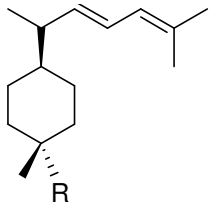
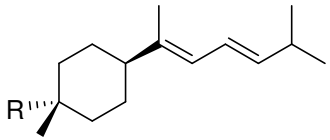
Names/ Structures	Sources	Activities	References
nitrogenous sesquiterpenes			
3-isocyanobisabolane-8,10-diene (**4**), 3-formamidobisabolane-8,10-diene (**5**)  (**4**); R = NC (**5**); R = NHCHO	*Halichondria* cf. *lendenfeldi*	N/A	(Kassuhlke *et al*., 1991)
3-isocyanotheonellin (**6**), 3-formamidotheonellin (**7**)  (**6**); R = NC (**7**); R = NHCHO	*Halichondria* cf. *lendenfeldi*	N/A	(Sullivan *et al*., 1986)

Table 1 (cont.)

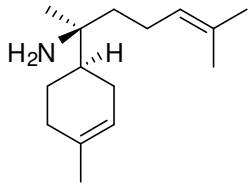
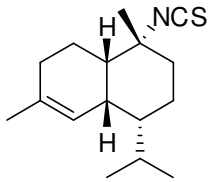
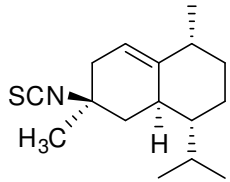
Names/ Structures	Sources	Activities	References
(6*R*,7*S*)-7-amino-7,8-dihydro-α-bisabolene (**8**) 	*Halichondria* sp.	- Antibacterial (inhibited 100 μg/disk against *S. aureus, B. subtilis* and *Vibrio anguillarum*) - Antifungal (MIC 5, 125 μg/mL against *C. albicans* and *Trichophyton mentagrophytes*, respectively)	(Sullivan *et al*., 1986)
10-isothiocyanato-4-amorphene (**9**) 	*Halichondria* sp.	- Antimalarial - Insecticidal - Herbicidal - Fungicidal	(Garson and Simpson, 2004; Erpenbeck *et al.,* 2005; Faulkner *et al*., 2004)
halipanicine (3-isothiocyanato-1(10)-cadinene) (**10**) 	*Halichondria panicca*	- Antimicrobial	(Nakamura *et al*., 1991)

Table 1 (cont.)

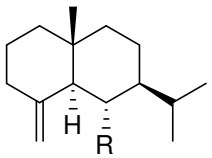
Names/ Structures	Sources	Activities	References
halichonadins B-D (**11-13**)  (**11**); R = $NHCO_2CH_3$ (**12**); R = NC (**13**); R = NH_2	*Halichondria* sp.	halichonadins B - Antibacterial (MIC 4.18 μg/mL against *Micrococcus luteus*) - Antifungal (MIC 4.18 μg/mL against *Cryptococcus neoformans*) halichonadins C - Antibacterial (MIC 0.52 μg/mL against *Micrococcus luteus*) - Antifungal (MIC 0.0625 μg/mL against *Cryptococcus neoformans*) halichonadins D - Antibacterial (MIC 16.7 μg/mL against *Micrococcus luteus*) - Antifungal (MIC 33.3 μg/mL against *Cryptococcus neoformans*)	(Ishiyama *et al*., 2005)

Table 1 (cont.)

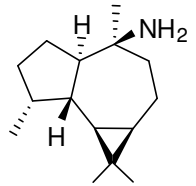
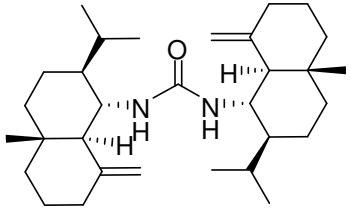
Names/ Structures	Sources	Activities	References
halichonadin F (**14**) 	*Halichondria* sp.	- Antibacterial (MIC 4 μg/mL against *Micrococcus luteus*) - Antifungal (MIC 16 μg/mL against *Cryptococcus neoformans* and *Trichophyton mentagrophytes*)	(Ishiyama *et al*., 2008)
nitrogenous sesquiterpenes derivertive			
halichonadin A (**15**) 	*Halichondria* sp.	- Antibacterial (MIC 16.7 μg/mL against *Micrococcus luteus*) - Antifungal (MIC 16.6 μg/mL against *Cryptococcus neoformans*)	(Ishiyama *et al*., 2005)

Table 1 (cont.)

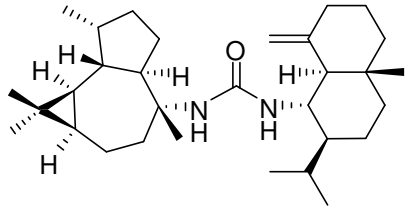
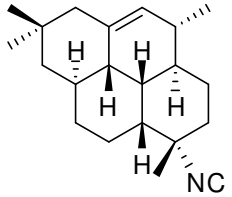
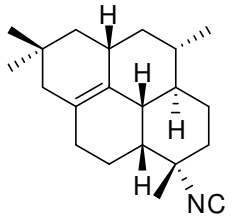
Names/ Structures	Sources	Activities	References
halichonadin E (**16**) 	*Halichondria* sp.	- Cytotoxic (IC_{50} 3.0 and 2.6 μg/mL against L1210 and KB, respectively)	(Kozawa *et al*., 2008)
nitrogenous diterpenes			
7-isocyano-1-cycloamphilectene (**17**) 	*Halichondria* sp.	N/A	(Molinski *et al*., 1987)
7-isocyano-11-cycloamphilectene (**18**) 	*Halichondria* sp.	N/A	(Molinski *et al*., 1987)

Table 1 (cont.)

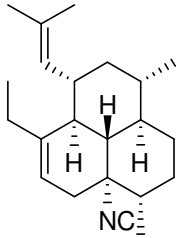
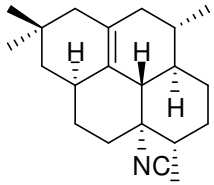
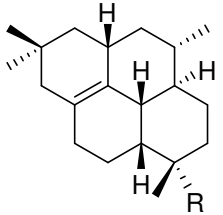
Names/ Structures	Sources	Activities	References
8-isocyano-l0,14-amphilectadiene (**19**) 	*Halichondria* sp.	- Antibacterial (inhibited 5 *μ*g/disk against *Staphylococcus aureus* and *Bacillus subtilis*)	(Molinski *et al*., 1987)
8-isocyano-1(12)-cycloamphilectene (**20**) 	*Halichondria* sp.	- Antibacterial (inhibited 5 *μ*g/disk against *Staphylococcus aureus* and *Bacillus subtilis*)	(Molinski *et al*., 1987)
formamido-substituted cycloamphilectenes (**21-22**)  (**21**); R = NHCHO, (**22**); R = NC	*Halichondria* sp.	N/A	(Garson and Simpson, 2004)

Table 1 (cont.)

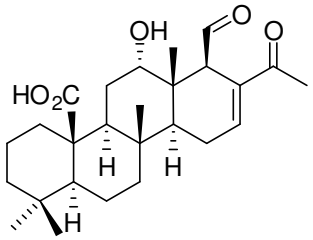
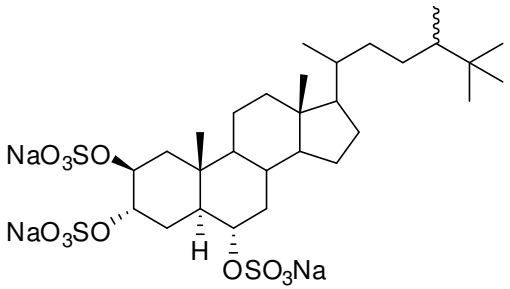
Names/ Structures	Sources	Activities	References
Sesterterpenes			
homosesterterpene (**23**) 	*Halichondria* sp.	N/A	(Nakagawa *et al*., 1987)
Steroids			
hilistanol sulfate (**24**) 	*Halichondria* cf. *moorei* Bergquist	- Antimicrobial - Hemolytic - Ichthyotoxic	(Fusetani *et al*., 1981)

Table 2 Polyethers/macrolides from the sponges of genus *Halichondria*

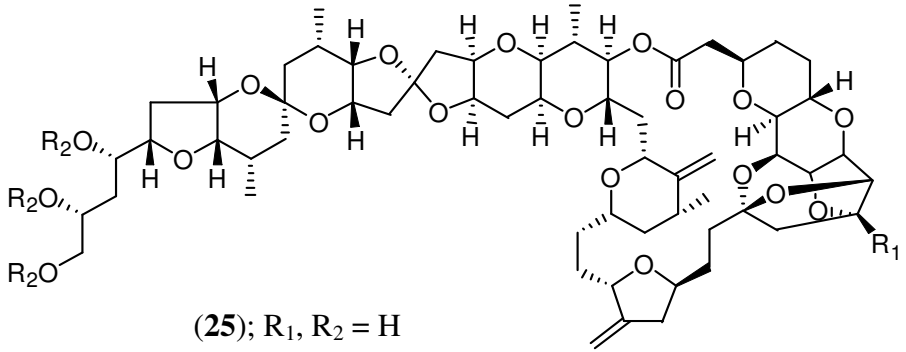
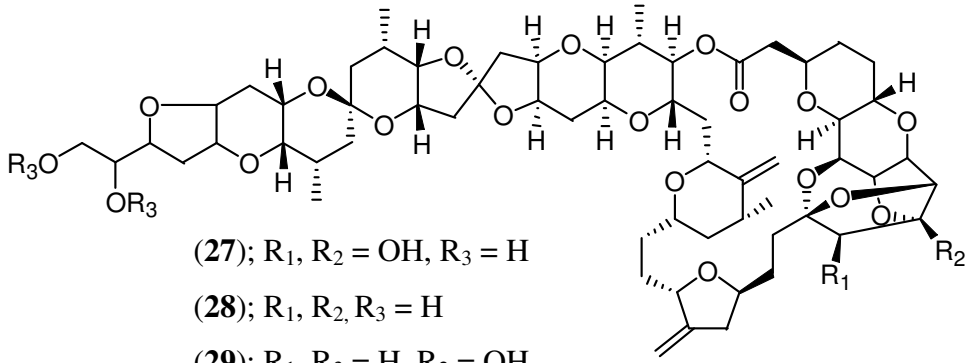
Names/ Structures	Sources	Activities	References
polyether macrolides			
halichondrin B (**25**), C (**26**)  (**25**); R_1, R_2 = H (**26**); R_1 = OH, R_2 = H	*Halichondria okadai* Kadota	halichondrin B - Cytotoxic (IC_{50} 0.3 nM and 0.093 ng/mL against L1210 and B-16, respectively) halichondrin C - Cytotoxic (IC_{50} 0.35 ng/mL against B-16)	(Hirata and Uemura, 1986)
homohalichondrin A-C (**27-29**)  (**27**); R_1, R_2 = OH, R_3 = H (**28**); R_1, R_2, R_3 = H (**29**); R_1, R_3 = H, R_2 = OH	*Halichondria okadai* Kadota	homohalichondrin A-C - Cytotoxic (IC_{50} 0.26, 0.1, 0.1 ng/mL against B-16, respectively)	(Hirata and Uemura, 1986)

Table 2 (cont.)

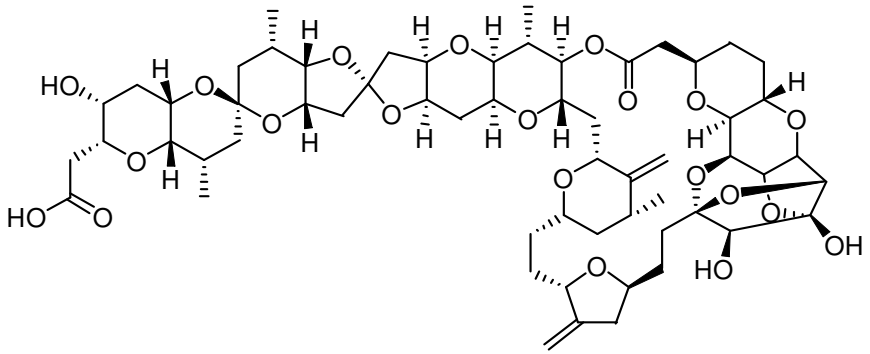
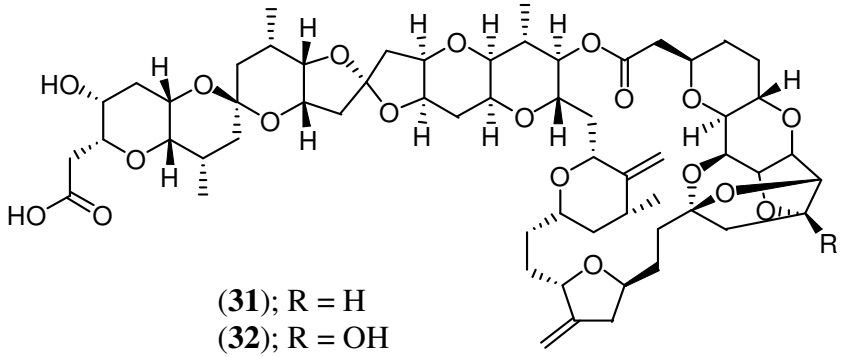
Names/ Structures	Sources	Activities	References
norhalichondrin A (**30**) 	*Halichondria okadai* Kadota	- Cytotoxic (IC_{50} 5 ng/mL against B-16)	(Uemura *et al*., 1985)
norhalichondrin B (**31**), C (**32**)  (**31**); R = H (**32**); R = OH	*Halichondria okadai* Kadota	norhalichondrin B, C - Antitumor (IC_{50} 5.2 ng/mL against B-16)	(Hirata and Uemura, 1986)

Table 2 (cont.)

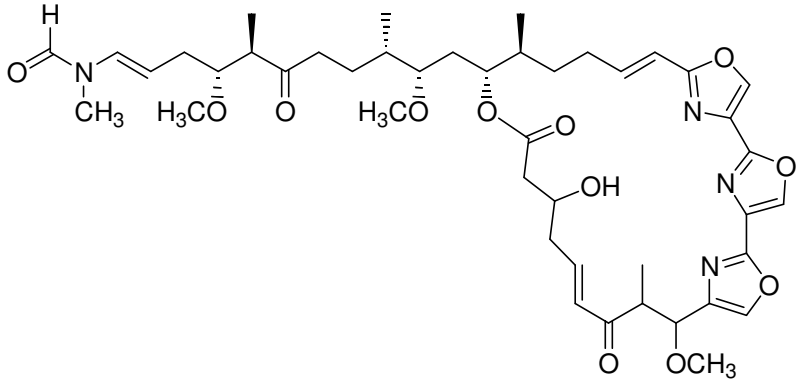
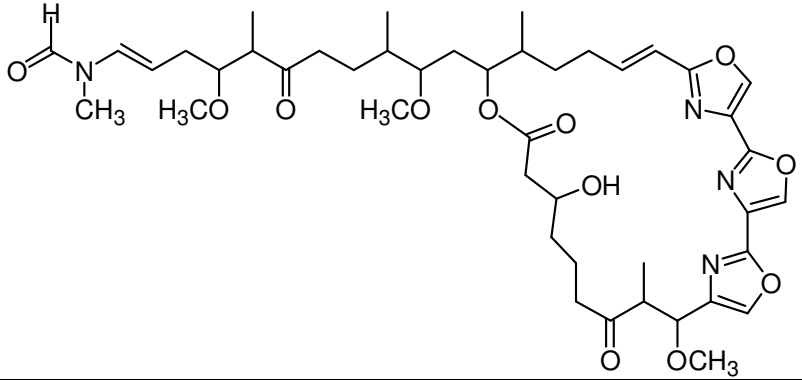
Names/ Structures	Sources	Activities	References
Macrolides			
halichondramide (**33**) 	*Halichondria* sp.	- Antifungal (MIC 0.2, 12.5 μg/mL against *Candida albicans* and *Trichophyton mentagrophytes*, respectively)	(Kernan and Faulkner, 1987)
dihydrohalichondramide (**34**) 	*Halichondria* sp.	- Antifungal (at 0.5 μg/disk against *Candida albicans*)	(Kernan *et al*., 1988)

Table 2 (cont.)

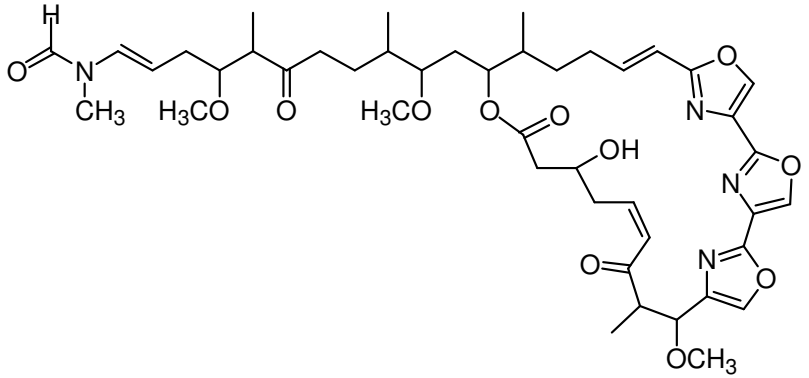
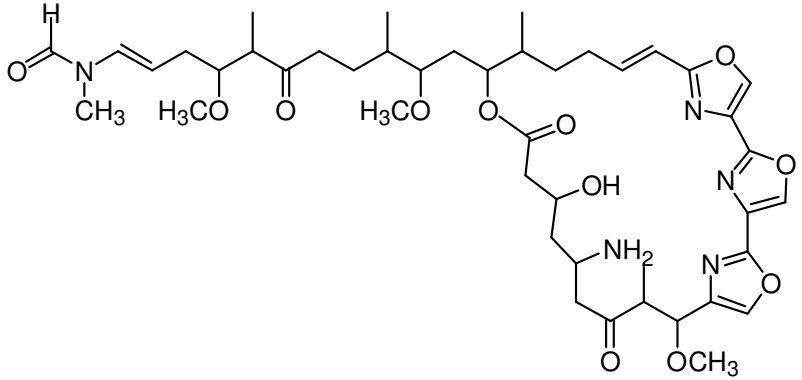
Names/ Structures	Sources	Activities	References
isohalichondramide (**35**) 	*Halichondria* sp.	- Antifungal (at 0.5 μg/disk against *Candida albicans*)	(Kernan *et al*., 1988)
halishigamide A (**36**) 	*Halichondria* sp.	- Cytotoxic (IC_{50} 0.0036 and 0.012 μg/mL against L1210 and KB, respectively) - Antifungal (MIC 0.1 μg/mL against *Trichophyton mentagrophytes*)	(Kobayashi *et al*., 1997)

Table 2 (cont.)

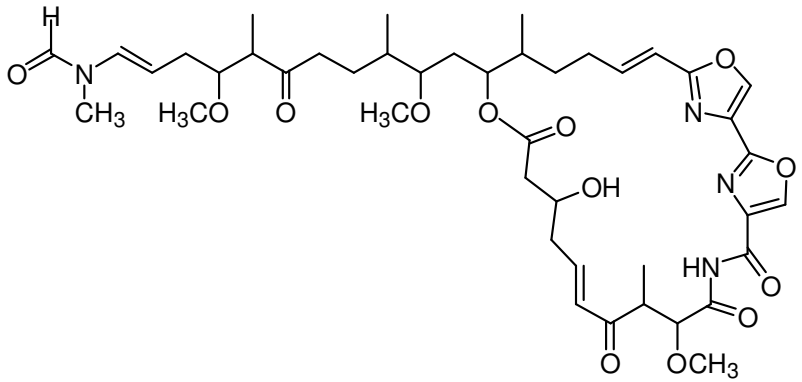
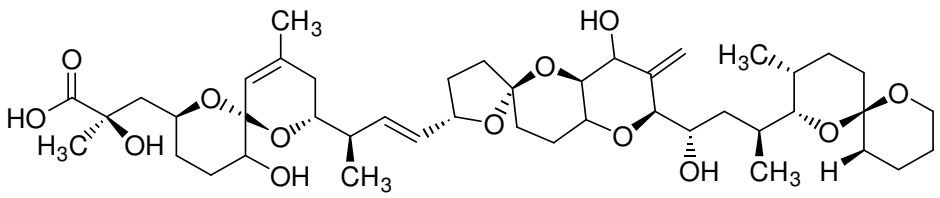
Names/ Structures	Sources	Activities	References
halishigamide B (**37**) 	*Halichondria* sp.	- Cytotoxic (IC_{50} 4.4 and 7.5 μg/mL against L1210 and KB, respectively) - Antifungal (MIC 25 μg/mL against *Trichophyton mentagrophytes*)	(Kobayashi *et al*., 1997)
Polyethers			
okadaic acid (**38**) 	*Halichondria okadai* kadota and *H. melonodocia*	- Cytotoxic (ED_{50} 1.7×10^{-3} and 1.7×10^{-2} against P-388 and L1210, respectively)	(Tachibana *et al*., 1981)

Table 3 Alkaloids from the sponges of genus *Halichondria*

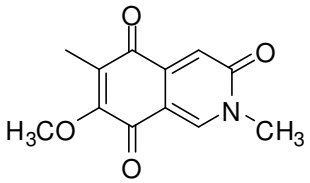
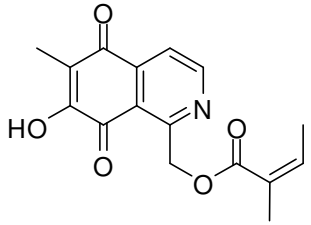
Names/ Structures	Sources	Activities	References
isoquinoline quinine alkaloids			
mimosamycin (**39**) 	*Halichondria* sp.	N/A	(Sung *et al*., 2006)
O-demethylrenierone (**40**) 	*Halichondria* sp.	N/A	(Sung *et al*., 2006)

Table 3 (cont.)

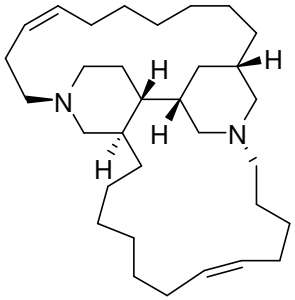
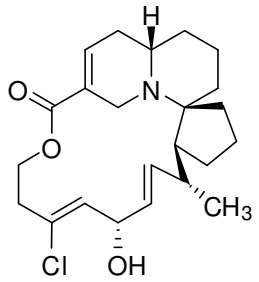
Names/ Structures	Sources	Activities	References
tetracyclic alkylbipiperidine alkaloid			
halichondramine (**41**) 	*Halichondria* sp.	N/A	(Chill *et al*., 2002)
cyclic polyketide alkaloid			
halichlorine (**42**) 	*Halichondria okadai* Kadota	- Antiatherosclerosis (IC_{50} 7 μg/mL inhibited VCAM-1)	(Kuramoto *et al*., 1996)

Table 4 Miscellaneous compounds from the sponges of genus *Halichondria*

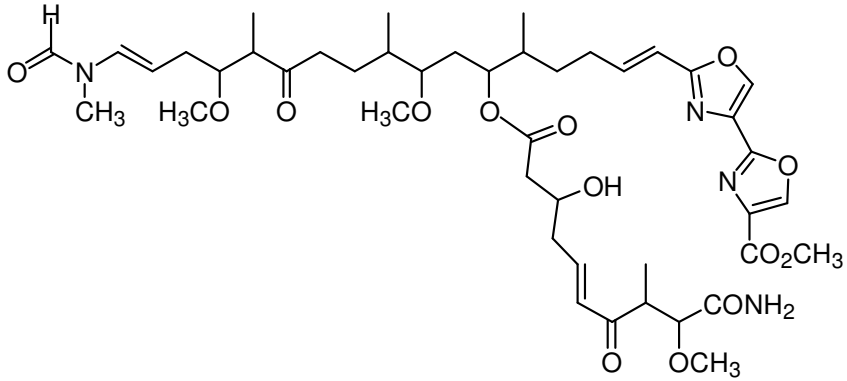
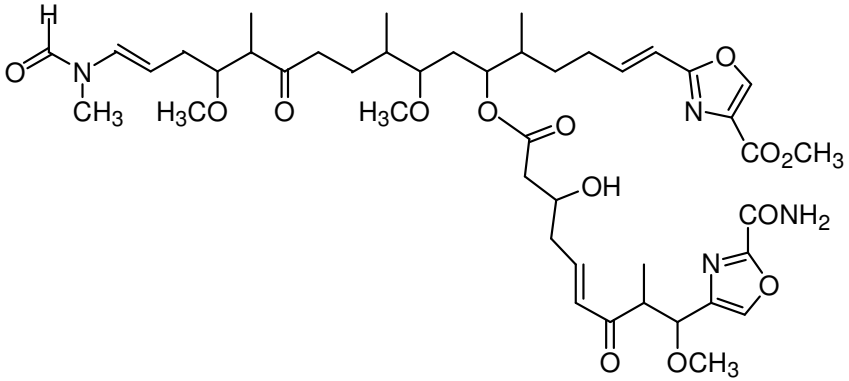
Names/ Structures	Sources	Activities	References
halishigamide C (**43**) 	*Halichondria* sp.	- Cytotoxic (IC_{50} 5.2 and 6.5 μg/mL against L1210 and KB, respectively) - Antifungal (MIC 25 μg/mL against *Trichophyton mentagrophytes*)	(Kobayashi *et al*., 1997)
halishigamide D (**44**) 	*Halichondria* sp.	- Cytotoxic (IC_{50} 1.1 and 1.8 μg/mL against L1210 and KB, respectively) - Antifungal (MIC 6.5 μg/mL against *Trichophyton mentagrophytes*)	(Kobayashi *et al*., 1997)

Table 4 (cont.)

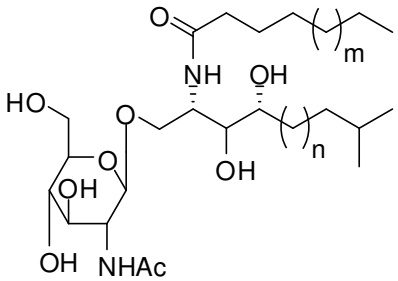
Names/ Structures	Sources	Activities	References
glycoshingolipids			
halicylindrosides A_1-A_4 (**45-48**)  (**45**); m = 16, n = 10 (**46**); m = 16, n = 11 (**47**); m = 17, n = 11 (**48**); m = 18, n = 11	*Halichondria cylindrata* and *H. panacea*	halicylindrosides A_1-A_4 - Antifungal against *Mortierella remanniana* at 250 μg/disk - Cytotoxic (at 6.8 μg/mL against P-388)	(Li *et al*., 1995)

Table 4 (cont.)

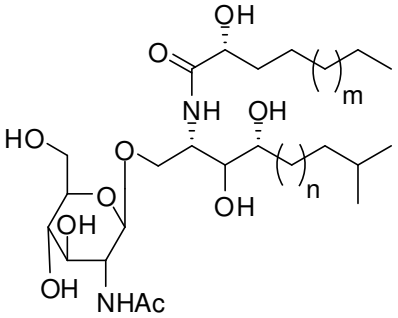
Names/ Structures	Sources	Activities	References
halicylindrosides B_5-B_{10} (**49-54**)  (**49**); m = 16, n = 8 (**50**); m = 17, n = 8 (**51**); m = 16, n = 9 (**52**); m = 16, n = 10 (**53**); m = 17, n = 9 (**54**); m = 16, n = 11	*Halichondria cylindrata* and *H. panacea*	halicylindrosides B_5-B_{10} - Antifungal against *Mortierella remanniana* at 250 μg/disk - Cytotoxic (at 6.8 μg/mL against P-388)	(Li *et al*., 1995)

Table 4 (cont.)

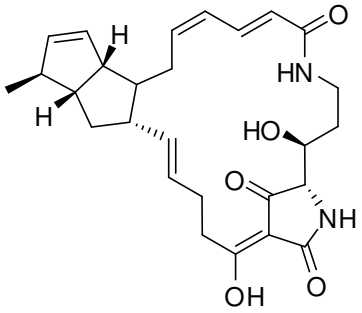
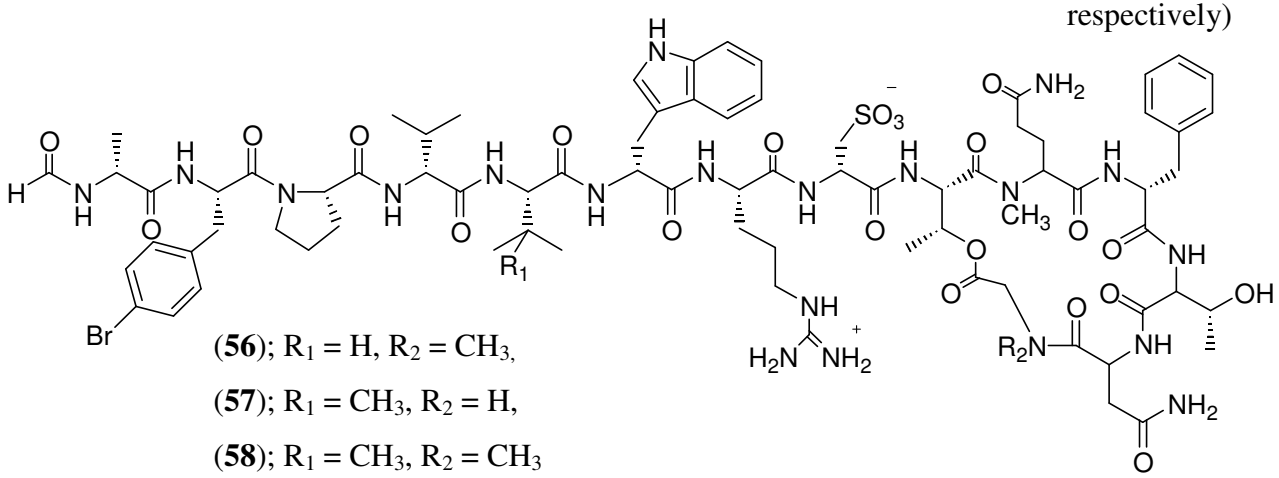
Names/ Structures	Sources	Activities	References
tetramic acid lactam			
cylindramide (**55**) 	*Halichondria cylindrata*	- Cytotoxic (IC_{50} 0.8 μg/mL against B16)	(Kanazawa *et al.*, 1993)

Table 4 (cont.)

Names/ Structures	Sources	Activities	References
tetradecapeptides			
halicylindramides A-C (**56-58**)	*Halichondria cylindrata*	halicylindramides A-C - Antifungal (at 7.5 μg/disk against *Mortierella ramanniana*) halicylindramides A and B,C - Cytotoxic (IC_{50} 0.54 and 0.2 μg/mL against P-388, respectively)	(Li *et al.,* 1995)



(**56**); R_1 = H, R_2 = CH_3,

(**57**); R_1 = CH_3, R_2 = H,

(**58**); R_1 = CH_3, R_2 = CH_3

Table 4 (cont.)

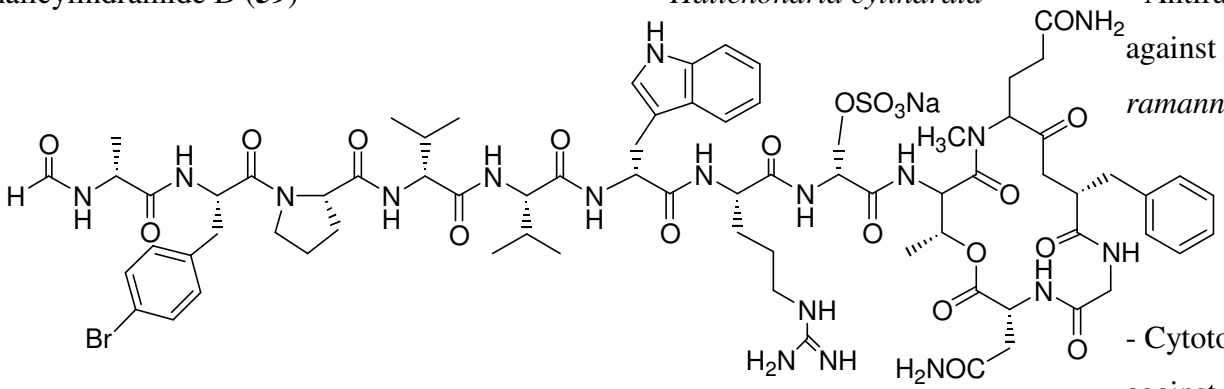
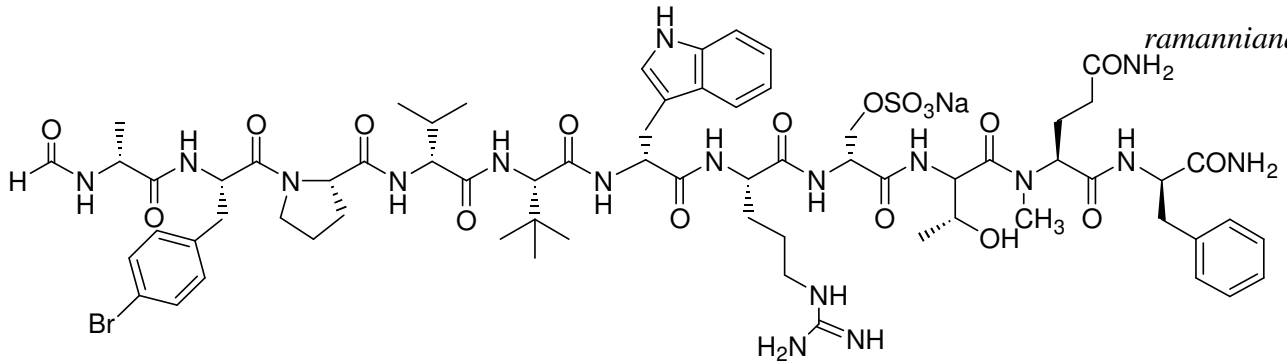
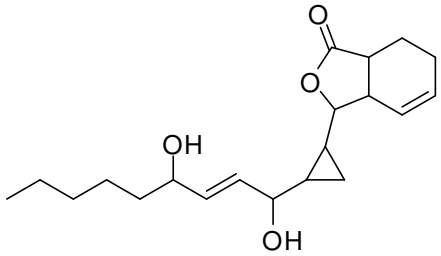
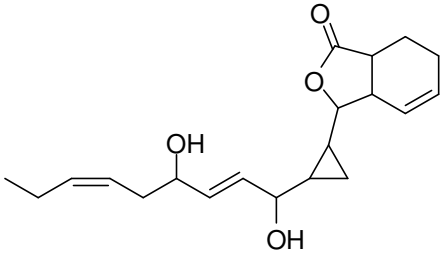
Names/ Structures	Sources	Activities	References
halicylindramide D (**59**) 	*Halichondria cylindrata*	- Antifungal (at 5 μg/disk against *Mortierella ramanniana*) - Cytotoxic (IC_{50} 2.1 μg/mL against P-388)	(Li *et al*., 1996)
halicylindramide E (**60**) 	*Halichondria cylindrata*	- Antifungal (at 5 μg/disk against *Mortierella ramanniana*)	(Li *et al*., 1996)

Table 4 (cont.)

Names/ Structures	Sources	Activities	References
fatty acid derivertives			
halichonlactone (**61**) 	*Halichondria okadai* kadota	- Exhibited weak inhibitory activity (IC_{50} = 630 μM) against 5-lipoxygenase of guinea pig polymorphonuclear leukocytes)	(Niwa *et al*., 1989)
neohalichonlactone (**62**) 	*Halichondria okadai* kadota	N/A	(Niwa *et al*., 1989)

1.3 Chemical constituents from the sponge of the genus *Aplysinopsis*

Many groups of compounds have been isolated from the sponge genus *Aplysinopsis* such as terpenoids and alkaloids.

1.3.1 Terpenoids (Table 5)

Sesterterpenoids were isolated from *Aplysinopsis digitata*, aplysinoplides A-C (**63-65**) (Ueoka *et al*., 2008). Aplyolide A (**66**), aplysolide A (**67a**) and B (**67b**) were isolated from *Aplysinopsis* sp. (Ungur and Kulcitki, 2009). Isodehydroluffariellolide (**68**) was isolated from *Fascaplysinopsis reticulata* (Jimenez *et al*., 1991) and luffariellolide (**69**) was isolated from *Fascaplysinopsis* sp. (Roll *et al*., 1988).

1.3.2 Alkaloids (Table 6)

Alkaloids were isolated from *Fascaplysinopsis* sp., fascaplysin (**70**) (Roll *et al*., 1988). Secofascaplysin A (**71**), homofascaplysin A cation (**72**), homofascaplysin B (**73**), homofascaplysin C (**74**), aplysinopsin (**75**), *N*3′-methylaplysinopsin (**76**), 6-bromoaplysinopsin (**77**), and 3′-deimino-3′-oxoaplysinopsin (**78**) were isolated from *Fascaplysinopsis reticulata* (Jimenez *et al*., 1991; Mancini *et al*., 2003).

Table 5 Terpenoids from the sponges of genus *Aplysinopsis*

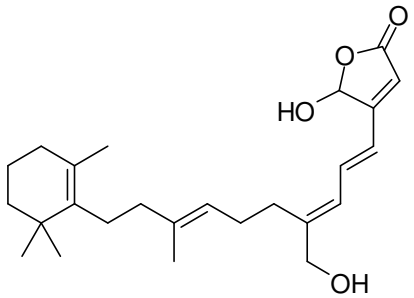
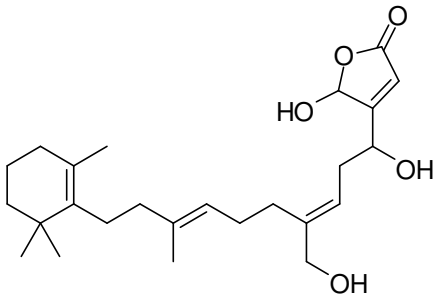
Names/ Structures	Sources	Activities	References
Sesterterpenoids			
aplysinoplides A (**63**) 	*Aplysinopsis digitata*	- Cytotoxic (IC_{50} 0.45 μg/mL against P-388)	(Ueoka *et al*., 2008)
aplysinoplides B (**64**) 	*Aplysinopsis digitata*	- Cytotoxic (IC_{50} 0.45 μg/mL against P-388)	(Ueoka *et al*., 2008)

Table 5 (cont.)

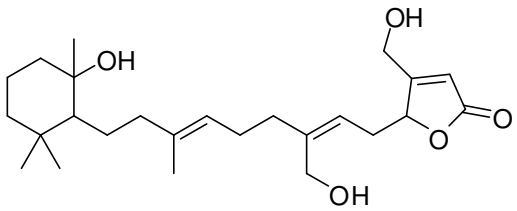
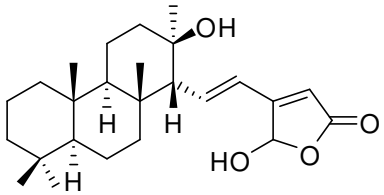
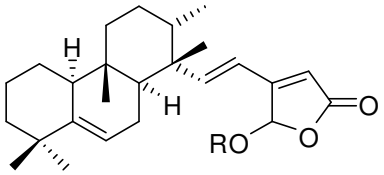
Names/ Structures	Sources	Activities	References
aplysinoplides C (**65**) 	*Aplysinopsis digitata*	- Cytotoxic (IC_{50} 11 μg/mL against P-388)	(Ueoka *et al*., 2008)
aplyolide A (**66**) 	*Aplysinopsis* sp.	- Antiinflammatory inhibited PLA_2 (IC_{50} = 10.50 μM)	(Crews *et al*., 1991; Ungur and Kulcitki, 2009)
aplysolide A (**67a**), B (**67b**)  (**67a,b**); R = H (α or β)	*Aplysinopsis* sp.	N/A	(Crews *et al*., 1991; Ungur and Kulcitki, 2009)

Table 5 (cont.)

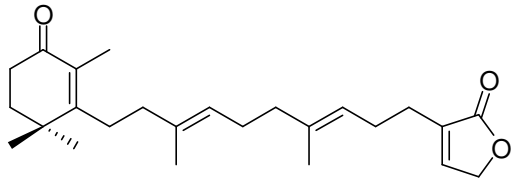
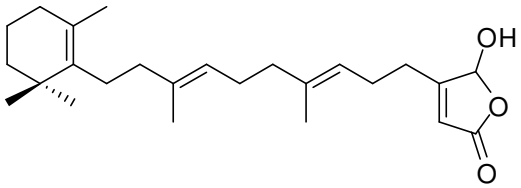
Names/ Structures	Sources	Activities	References
isodehydroluffariellolide (**68**) 	*Fascaplysinopsis reticulata*	- Antiviral (at 1 mg/mL inhibited 81% against reverse transcriptase)	(Jimenez *et al*., 1991)
luffariellolide (**69**) 	*Fascaplysinopsis* sp.	- Cytotoxic (IC_{50} = 3.3 μg/mL against L1210)	(Roll *et al*., 1988)

Table 6 Alkaloids from the sponge of genus *Aplysinopsis*

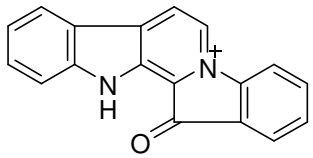
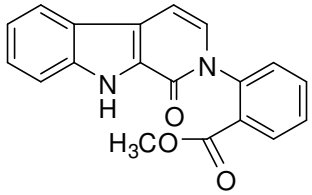
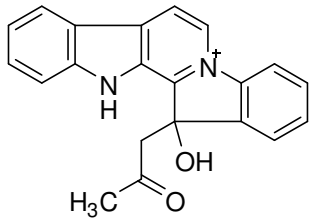
Names/ Structures	Sources	Activities	References
fascaplysin (**70**) 	*Fascaplysinopsis* sp.	- Cytotoxic (IC_{50} = 0.2 μg/mL against L1210)	(Roll *et al*., 1988)
secofascaplysin A (**71**) 	*Fascaplysinopsis reticulata*	N/A	(Jimenez *et al*., 1991)
homofascaplysin A cation (**72**) 	*Fascaplysinopsis reticulata*	- Antiviral (at 1 mg/mL inhibited 94% against reverse transcriptase)	(Jimenez *et al*., 1991)

Table 6 (cont.)

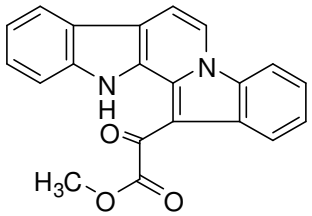
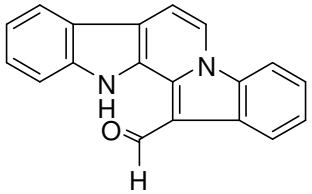
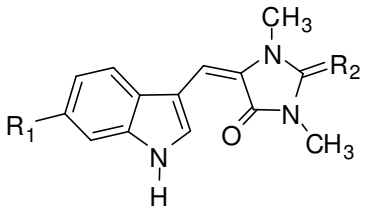
Names/ Structures	Sources	Activities	References
homofascaplysin B (**73**) 	*Fascaplysinopsis reticulata*	N/A	(Jimenez *et al*., 1991)
homofascaplysin C (**74**) 	*Fascaplysinopsis reticulata*	N/A	(Jimenez *et al*., 1991)

Table 6 (cont.)

Names/ Structures	Sources	Activities	References
aplysinopsin (**75**), *N*3´-methylaplysinopsin (**76**), 6-bromoaplysinopsin (**77**) and 3´-deimino-3´-oxoaplysinopsin (**78**)  (**75**); R_1 = H, R_2 = NH (**76**); R_1 = H, R_2 = NCH_3 (**77**); R_1 = Br, R_2 = NH (**78**); R_1 = H, R_2 = O	*Aplysinopsis reticulata*	N/A	(Mancini *et al*., 2003)

1.4 Objectives

This research is studying on the chemical constituents from Thai sponges of the *Halichondria* sp. (Class Demospongiae, Order Halichondrida, Family Halichondriidae) and *Aplysinopsis* sp. (Class Demospongiae, Order Dictyoceratida, Family Thorectidae). The sponge *Halichondria* sp. was collected by using scuba diving from Koh Kra, Nakhon Si Thammarat Province (Figure 1, Page 37) and sponge *Aplysinopsis* sp. was collected by using scuba diving from Koh Ha, Krabi Province (Figure 2, Page 37). The preliminary screening of methanolic extract from the sponges *Halichondria* sp. and *Aplysinopsis* sp. showed the potent activity with an IC_{50} of 0.405 and 2.58 μg/mL against *Plasmodium falciparum* K1 strain, respectively. Both species were selected to investigate its chemical constituents. The objectives of this investigation are therefore as the followings;

1) to isolate and purify the chemical constituents from the sponges *Halichondria* sp. and *Aplysinopsis* sp.

2) to elucidate the chemical structures of pure compounds, and

3) to study biological activity of pure compounds as mentioned in (2)

CHAPTER 2

EXPERIMENTAL

2.1 General experimental procedures

Solvents for column chromatography and thin layer chromatography (TLC) were commercial grade and were redistilled prior to use. Thin layer chromatography was performed on Merck® pre-coated silica gel 60 F_{254} plates (layer thickness 0.20 mm), and spots were detected under UV light (254 nm) and upon spraying with anisaldehyde reagent. Quick column chromatography was carried out using Merck® silica gel 60 (particle size 0.04-0.06 mm, 230-400 mesh ASTM). The optical rotation was determined on a Perkin-Elmer 341 polarimeter (Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

High performance liquid chromatography (HPLC) was performed on a Waters® 600E mulisolvent delivery system connected to Waters® 484 tunable absorbance detector. This was equipped with a Rheodyne® 7125 injector port. Gas chromatography-mass spectrometry (GC-MS) was performed on an HP 5890 gas chromatograph coupled with an HP 5972 mass selective detector. The column (30 m × 0.32 mm, 0.25 μm film thickness) was HP-1, coated with polydimethylsiloxane (Hewlett Packard®). Helium (TIG) was used as the carrier gas at a flow rate of 1 mL/min. The programmed column temperature was ramped from 180°C to 300°C at a route of 10°C/min, and hold at 300°C for an additional 20 min. The temperature at injector port was 280°C, and at detector port was 300°C. Detection was based on

electron ionization (EI) mass spectrometry. Peak identification was carried out by comparison with those available in the Wiley libraries.

Ultraviolet (UV) adsorption spectra were obtained from a Genesys™ 6 spectrophotometer. Infrared (IR) adsorption spectra were recorded on a Jasco® IR-810 infrared spectrometer (all at Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University). Proton and carbon-13 nuclear magnetic resonance (^{1}H and ^{13}C NMR) spectra were recorded on a Fourier Transform NMR, Varian® Unity Inova 500 spectrometer. The chemical shifts (ppm) were referred to the solvent peaks (benzene-d_6; δ_H 7.15 for residual C_6D_5H, and δ_C 128.0; and chloroform-*d*; δ_H 7.25 for residual $CHCl_3$, and δ_C 77.0). The EI-MS and ESI-TOF-MS were measured on a MAT 95 XL and Micromass® LCT mass spectrometer, respectively (all at Scientific Equipment Center, Prince of Songkla University).

2.2 Sponge material

2.2.1 *Halichondria* sp.

The sponge was collected by scuba diving from Koh Kra, Nakhon Si Thammarat Province, Thailand, in 2005. The sponge material was preserved in ice-chest, then at -20°C upon arrival at the laboratory. The sponge was identified as *Halichondria* sp. (Phylum Porifera Class Demospongiae, Order Halichondrida, Family Halichondriidae) by Dr. Somchai Bussarawit, the Phuket Marine Biology Center, Phuket, Thailand. The sponge voucher specimen (AP 05-001-01) was preserved in 70% ethanolic solution and was deposited at Department of

Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University.

Figure 1 The sponge *Halichondria* sp.

2.2.2 *Aplysinopsis* sp.

The sponge was collected by scuba diving from Koh Ha, Krabi Province, Thailand, in 2007. The sponge material was preserved in ice-chest, then at -20°C upon arrival at the laboratory. The sponge was identified as *Aplysinopsis* sp. (Phylum Porifera Class Demospongiae, Order Dictyoceratida, Family Thorectidae) by Dr. Somchai Bussarawit, the Phuket Marine Biology Center, Phuket, Thailand. The sponge voucher specimen (AP 07-012-03) was preserved in 70% ethanolic solution and was deposited at Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University.

Figure 2 The sponge *Aplysinopsis* sp.

2.3 Bioactivity determination

2.3.1 Antimalarial activity

The antimalarial activity determination was serviced by Central Bioassay Lab, National Center for Genetic Engineering and Biotechnology, Pathumthani. The parasite *Plasmodium falciparum* (K1, multidrug resistant strain) was cultured continuously according to the method of Trager and Jensen (1976). Quantitative assessment of antimalarial activity *in vitro* was determined by means of the microculture radioisotope technique based on the method described by Desjardins *et al* (1979) and modified by Jongrungruangchok *et al* (2004).

Briefly, a mixture of 200 μL of 1.5% of erythrocytes with 1% parasitemia at the early ring stage was pre-exposed to 25 μL of the medium containing a test sample dissolved in DMSO (0.1% final concentration) for 24 hours employing the incubation conditions described above. Subsequently, 25 μL of [^{3}H]-hypoxanthine in culture medium was added to each well and plates were incubated for additional 24 hours. Levels of incorporated radioactively labeled hypoxanthine were determined using the TopCount microplate scintillation counter. The activity was reported in IC_{50} using dihydroartemisinin (IC_{50} 3.7 nM) as standard reference.

2.3.2 Antitubercular activity

The antitubercular activity was assessed against *Mycobacterium tuberculosis* H_{37}Ra using the green fluorescent protein microplate assay (GFPMA) (Changsen *et al*., 2003) with the service provided by the Central Bioassay Lab, National Center for Genetic Engineering and Biotechnology, Pathumthani.

Briefly, testing was performed in black, clear-bottom, 96-well microplates (black view plates; Packard Instrument Company, Meriden, Conn.). Frozen $H_{37}Ra$ was thawed, sonicated for 15 s and cultured at 37°C with shaking in 200 mL of 7H9GTw-kanamycin until the turbidity reached 50 to 70 Klett units. The cell was pelleted, washed with PBS and then suspended in 20 mL of PBS buffer. Aliquots were stored at -80°C for up to 2 to 3 months. Sample solutions were prepared in dimethyl sulfoxide and were further diluted in 7H12 broth (7H9 broth supplemented with 4 μg of catalase per mL, 5 mg of bovine serum albumin per mL, 5.6 μg of palmitic acid per mL, and 1 mg of casitone per mL) supplemented with 0.2% vol/vol glycerol (7H12G). Frozen inocula were diluted in 7H12G to make final bacterial densities of 5×10^4 to 5×10^5 CFU/mL. The MIC values of the standard antituberculosis compounds, rifampicin, streptomycin, isoniazid, and ofloxacin, were 0.003-0.012, 0.156-0.313, 0.023-0.046, and 0.391-0.781 μM, respectively.

2.3.3 Antimicrobial activity

The antimicrobial activity determination was determined using the disc diffusion method (Lorian, 1996; Salie *et al*., 1996).

Mueller Hinton Agar (MHA, Difco™) was used for culturing the bacteria, and Sabouraud Dextrose Agar (SDA, Difco™) was used for culturing the fungus. The targeted microorganisms in this study are *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Trichophyton rubrum* DMST 30263, and *Candida albicans* TISTR 5779. The bacteria were preinoculated on MHA (35°C, 24 hr.), whereas the fungus was cultured on SDA

(35°C, 48 hr.). A suspension of microbe equivalent to 0.5 McFarland suspensions was prepared and used in the disc diffusion protocol.

Upon the swabed agar surface (aseptic technique), discs containing extract or standard reagent were placed. The plates were incubated at 35°C for 18 hours (for bacteria) and 48 hours (for fungus). The diameter of clear zone were reported in mm. The standard drug referred to tetracycline (22.3, 21.8 mm against *B. subtilis* and *S. aureus*, respectively), norfloxacin (33.7 mm against *E. coli*), and amphotericin B (27.1, 19.4 mm against *T. rubrum* and *C. albicans*, respectively).

2.3.4 Cytotoxic activity

The cytotoxic determinations were kindly supported by Assist. Prof. Dr. Supreeya Yuenyongsawad and Miss Siriporn Kittiwisut of Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University. The cell line used in this test was MCF-7 (breast adenocarcinoma). The cytotoxic activity was determined using sulphorhodamine B (SRB) assay (Skehan *et al*., 1990).

Briefly, cells at a logarithmic growth phase were harvested and diluted to 10^5 cells/mL with fresh DMEM/F-12 (with glutamine) supplemented with 10% heat-inactivated fetal bovine serum, 50 IU/mL penicillin G sodium, 50 μg/mL streptomycin sulphate, and 0.125 μg/mL amphotericin B. The cells are seeded as monolayered cells in a 96-well microplate. Plates were incubated at 37°C under 5% CO_2 atmosphere for 24 hours. The culture was treated with sample solutions (DMSO or medium 0.0008-5 mg/mL), and the incubation was continued for additional six days. After the incubation period, cells were fixed with 40% trichloroacetic acid,

washed with water, and air-dried. The plates were stained with 0.4% sulforhodamine B (SRB) in 1% acetic acid for 30 min. SRB was then removed with 1% acetic acid. Plates were air-dried before bound dye was solubilized with 10 mM Tris base. Optical density was read on a microtiter plate reader (492 nm). The activity was reported in IC_{50} using camptothecin (IC_{50} 0.27 μM) as the standard drug.

2.4 Isolation and purification

2.4.1 *Halichondria* sp.

The preliminary screening of methanolic extract from the sponge *Halichondria* sp. showed the potent antimalarial activity with IC_{50} of 0.405 μg/mL against *P. falciparum*. The freeze-dried sponge (252.30 g) was macerated with CH_3OH (3 L×4). The crude extract (68.83 g) was partitioned to yield hexane- (2.80 g), CH_2Cl_2- (2.74 g), and *n*-butanol-extracts (14.16 g).

The separation of the hexane extract was done using chromatographic technique over a SiO_2 column, eluted with 10% to 80% EtOAc in petroleum ether. Compounds **1** and **2** were co-crystallized as white needles (317 mg, 15.85% of hexane fraction). The mixtures were analyzed by GC-MS. The temperature programmed was 180-300°C at 10°C/min, 20 min hold. The injector temperature was 280°C and the detector temperature was 300°C. The carrier gas was helium at a flow rate 1 mL/min.

The separations of the CH_2Cl_2- and *n*-butanol-extracts were done using chromatographic techniques. But the separations were not successful. Because most of compounds stuck on stationary phase.

2.4.2 *Aplysinopsis* sp.

The preliminary screening of methanolic extract from the sponge *Aplysinopsis* sp. showed potent antimalarial activity with IC_{50} of 2.58 μg/mL against *P. falciparum*. The freeze-dried sponge (123.67 g) was extracted with a series of solvents to yield hexane-, CH_2Cl_2- and methanol-extracts (0.99, 0.50 and 6.39 g, respectively). The hexane- and CH_2Cl_2-extract showed antimalarial activity against *P. falciparum* (IC_{50} 2.83 and 3.31 μg/mL, respectively).

The hexane extract (0.80 g) was separated by using chromatographic technique over a SiO_2 column, eluted with 5% to 100% EtOAc in petroleum ether. Compound **3** was crystallized as white needles (455.8 mg, 56.98% of hexane fraction).

The CH_2Cl_2 extract (0.40 g) was separated using chromatographic technique over a SiO_2 column, eluted with 20% to 100% CH_2Cl_2 in petroleum ether and 5% to 100% EtOAc in CH_2Cl_2. The pooled major fraction was then chromatographed over a reverse phase HPLC C18 column (VertiSep™ IRS, 10 μm, 250×10 mm) with isocratic 20% aq CH_3CN (5 mL/min, 254 nm) to yield compounds **4** (0.5 mg), **5** (7.5 mg), **6** (1.2 mg), and **7** (1.9 mg) (t_R 13.3, 14.8, 17.1 and 24.6 min, respectively).

Heteronemin (**3**): white needles; $[\alpha]_D$: -37.6° (c = 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 207 (3.39); IR (thin film) ν_{max} 3500, 1740, 1235 cm^{-1}; ^1H and ^{13}C NMR (500 MHz for ^1H; $CDCl_3$) see Table 7; ESIMS *m/z* (% relative intensity) 511 ($[M+Na]^+$, 100), 429 (4), 369 (30).

12-Deacetyl-12,18-di-*epi*-scalaradial (**4**): white solid; $[\alpha]_D$: -135.0° (*c* = 0.02, MeOH); UV (MeOH) λ_{max} (log ε) 225 (3.77); IR (thin film) ν_{max} 3410, 1720, 1680 cm^{-1}; ^1H and ^{13}C NMR (500 MHz for ^1H; $CDCl_3$) see Table 8; EIMS *m/z* 386 ($[M]^+$, 7), 385 (15), 354 (20), 277 (41), 276 (100).

12-Deacetyl-12-*epi*-scalaradial (**5**): white solid; $[\alpha]_D$: +14.8° (*c* = 0.27, MeOH); UV (MeOH) λ_{max} (log ε) 219 (3.93); IR (thin film) ν_{max} 3450, 1715, 1690 cm^{-1}; ^1H and ^{13}C NMR (500 MHz for ^1H; $CDCl_3$) see Table 9; EIMS *m/z* 386 ($[M]^+$, 4), 385 (12), 367 (18), 357 (47), 274 (75), 191 (100).

12-Deacetyl-12-*epi*-19-deoxyscalarin (**6**): white solid; $[\alpha]_D$: -35.2° (*c* = 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 222 (3.98); IR (thin film) ν_{max} 3420, 1740 cm^{-1}; ^1H and ^{13}C NMR (500 MHz for ^1H; $CDCl_3$) see Table 10; EIMS *m/z* 386 ($[M]^+$, 3), 385 (11), 340 (12), 339 (42), 191 (100).

12-*epi*-Scalaradial (**7**): white solid; $[\alpha]_D$: +38.8° (*c* = 0.09, MeOH); UV (MeOH) λ_{max} (log ε) 228 (3.34); IR (thin film) ν_{max} 1740, 1240 cm^{-1}; ^1H and ^{13}C NMR (500 MHz for ^1H; $CDCl_3$) see Table 11; EIMS *m/z* 428 ($[M]^+$, 4), 427 (7), 415 (15), 367 (20), 340 (32), 339 (100).

CHAPTER 3

RESULTS AND DISCUSSION

In the investigation of chemical constituents from Thai marine sponges, *Halichondria* sp. and *Aplysinopsis* sp. led to obtain sterol mixture from *Halichondria* and five sesterterpenes from *Aplysinopsis*.

3.1 *Halichondria* sp.

3.1.1 The isolation

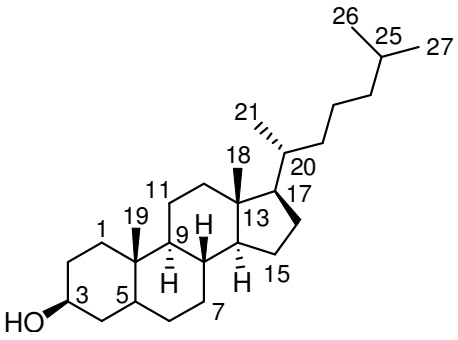
The hexane extract of the sponge *Halichondria* sp. was purified to yield a mixture of two steroids identified as dihydrocholesterol (**1**) and (3*β*, 24S)-stigmast-5-en-3-ol (**2**).

3.1.2 The structure elucidation

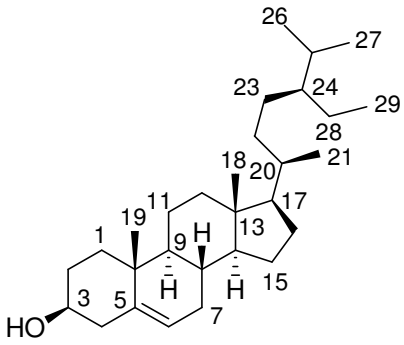
3.1.2.1 Dihydrocholesterol and (3*β*, 24S) stigmast-5-en-3-ol (1 and 2) mixture

The ^{1}H NMR spectrum (Figure 3) of **1** and **2** showed characteristic signals of steroid nucleus. These include the signals of H-3 at δ 3.39, H-6 at δ 5.36, and cluster of aliphatic part of steroid at δ 0.48-2.26. The proton intensity ratio of signal at δ 5.36 and signal at δ 3.39 is 1:2 indicated that this is ^{1}H NMR spectrum of a mixture. Due to the complicated NMR spectrum, the identification **1** and **2** was accomplished by means of GC-MS, instead. GC chromatogram of **1** and **2** (Figure 4) confirmed that the mixture was composed of two major components. Compound **1**,

eluted at t_R 15.42 min, showed a molecular peak at *m/z* 388 (Figure 5), with a fragmentation consistent to that of dihydrocholesterol (Wiley libraries), whereas **2**, eluted at t_R 16.85 min, did at *m/z* 414 (Figure 6), and consistent with the fragmentation of (3β, 24S) stigmast-5-en-3-ol.



1



2

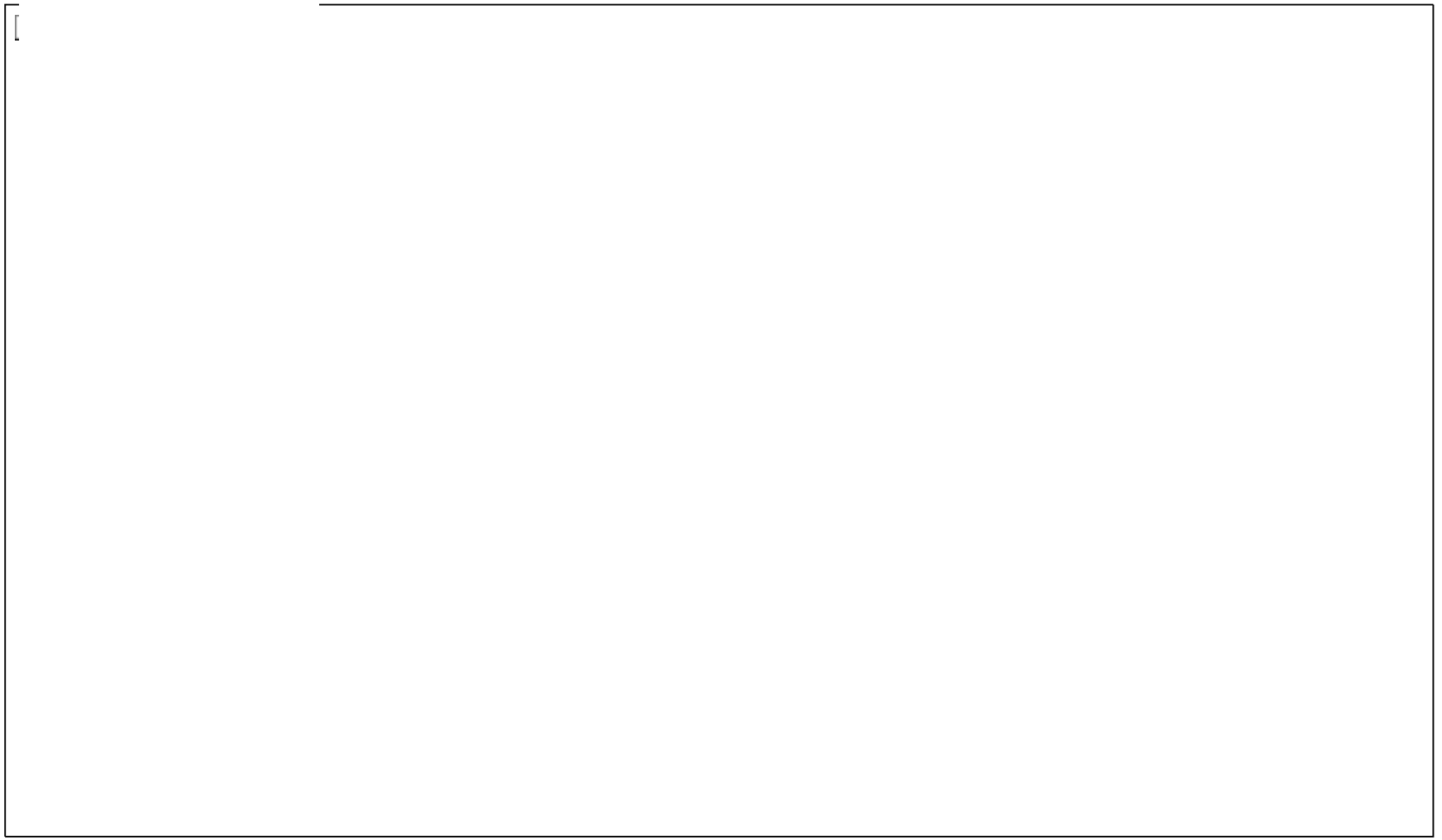


Figure 3 ^{1}H NMR spectrum of **1** and **2** (C_6D_6; 500 MHz)

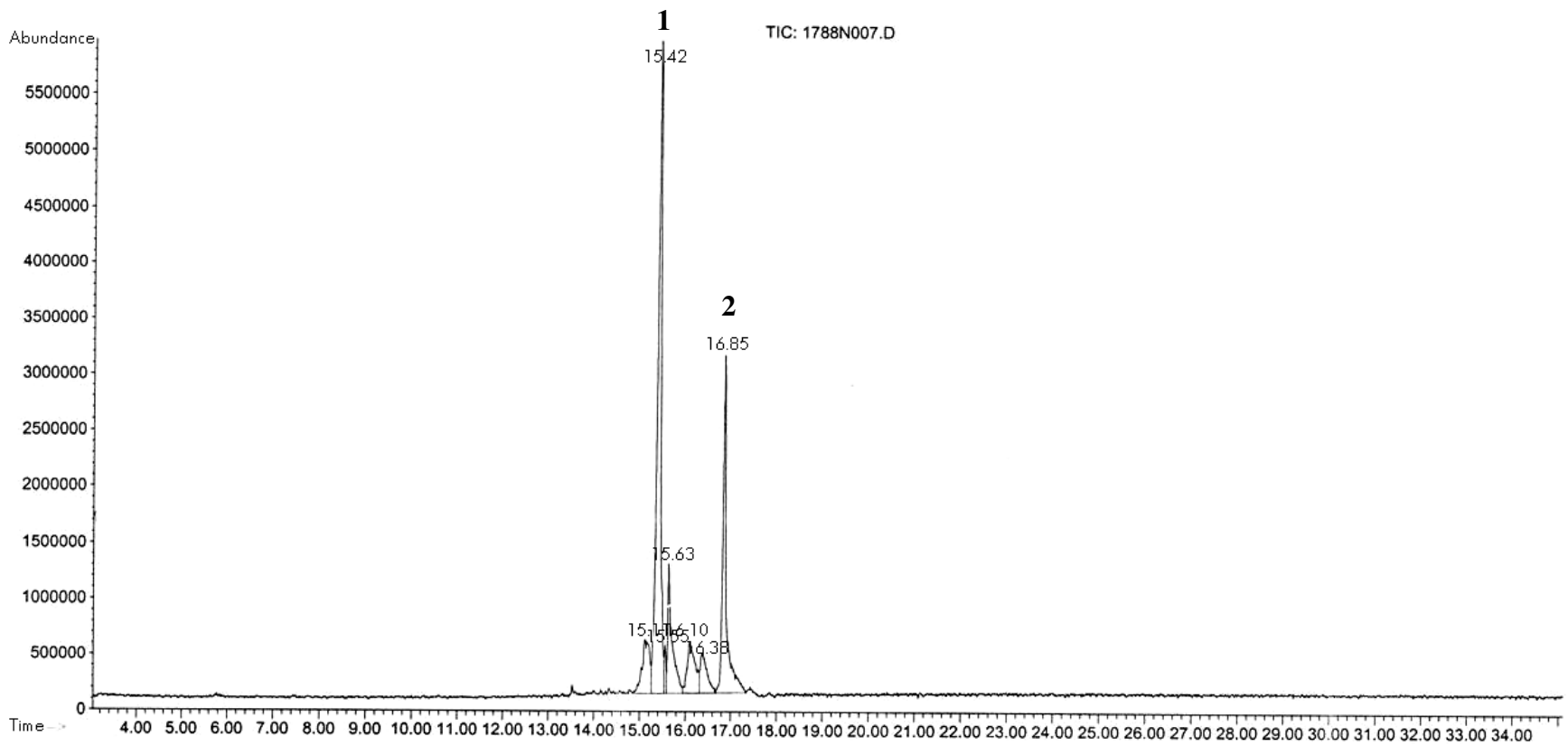


Figure 4 GC chromatogram of **1** and **2**

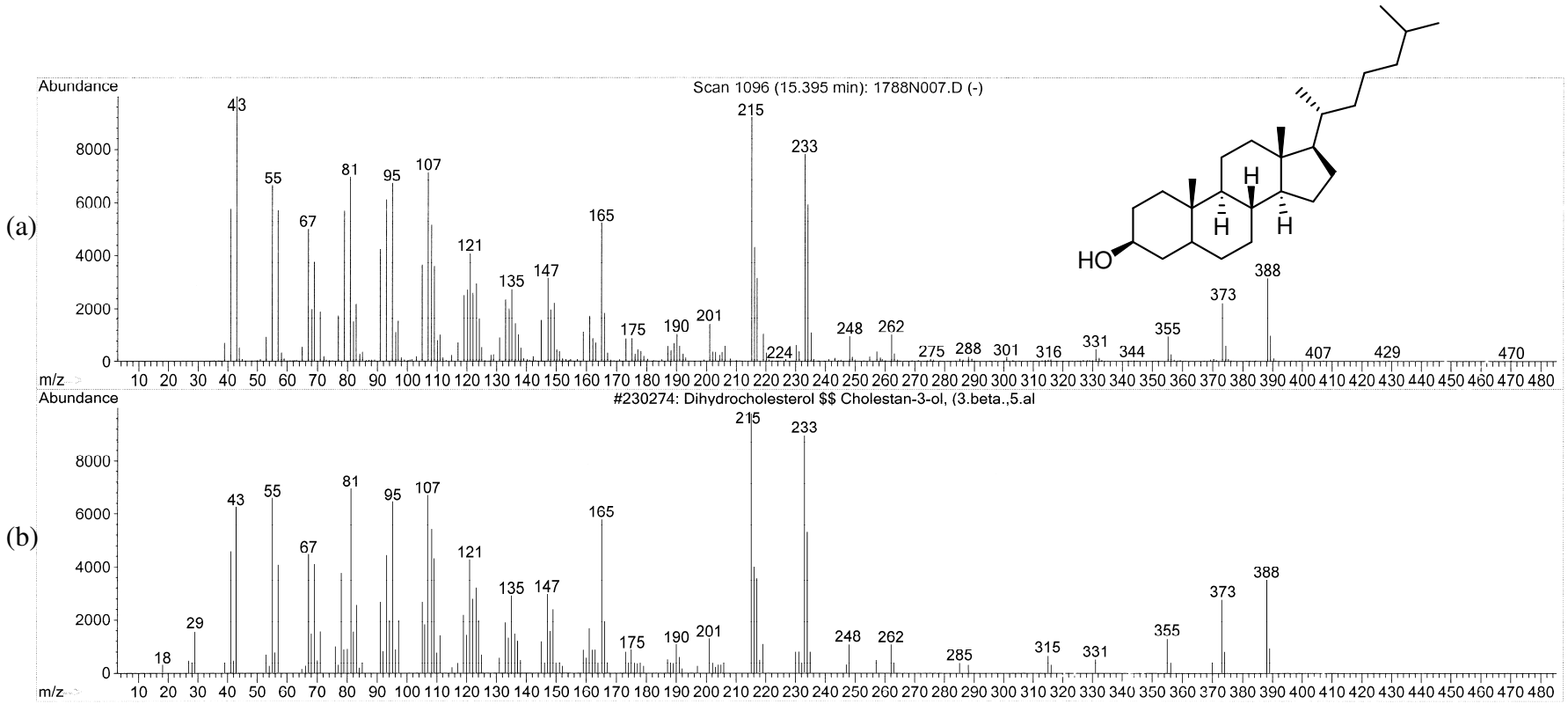


Figure 5 Mass spectra of (a) **1** and (b) dihydrocholesterol

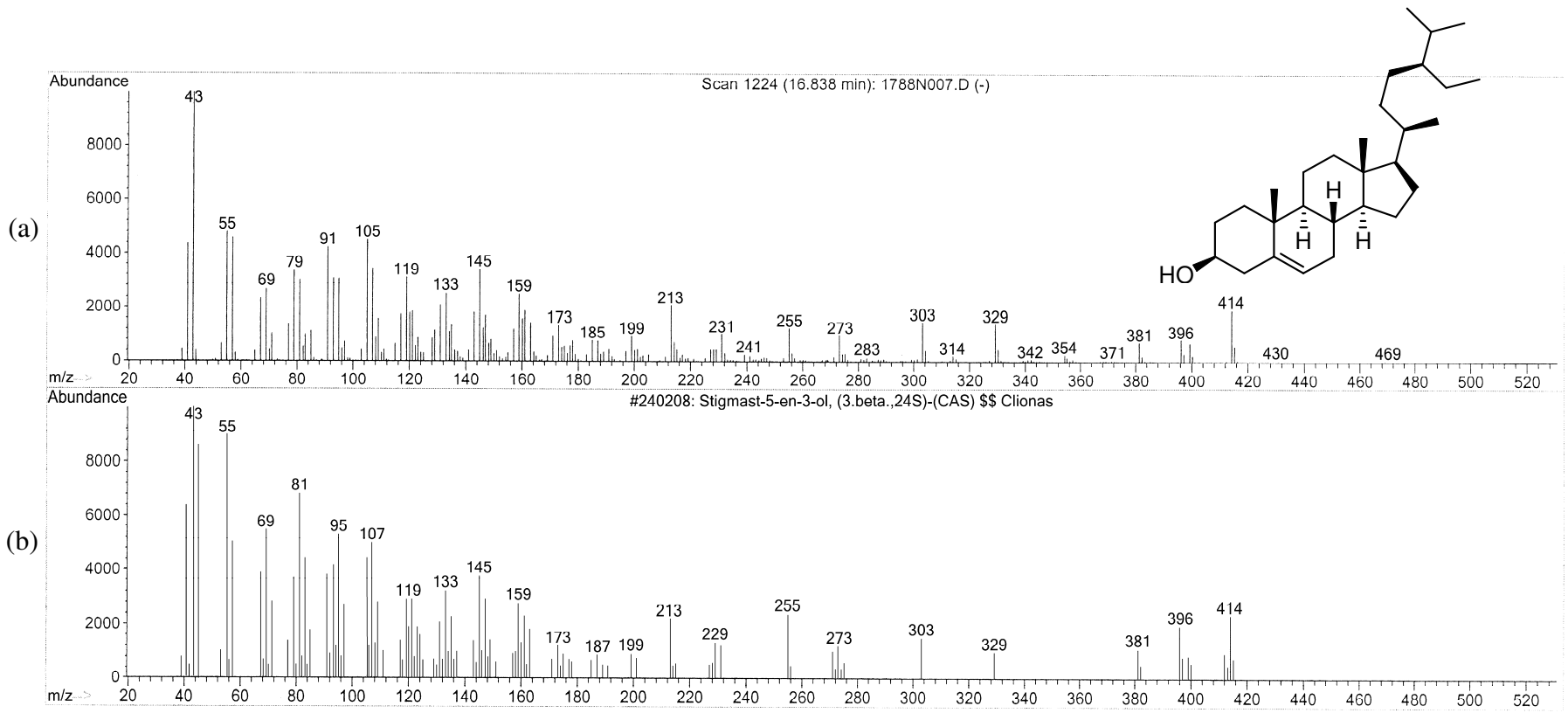


Figure 6 Mass spectra of (a) **2** and (b) (3*β*, 24S) stigmast-5-en-3-ol

3.2 *Aplysinopsis* sp.

3.2.1 The isolation

The sponge (123.67 g) was extracted to yield hexane-, CH_2Cl_2- and methanol-extracts (0.99, 0.50, and 6.39 g, respectively). The active hexane-extract (IC_{50} 2.83 μg/mL against *P. falciparum*) was purified by column chromatographic technique to yield heteronemin (**3**) (455.8 mg). The active CH_2Cl_2-extract (IC_{50} 3.31 μg/mL against *P. falciparum*) was separated to yield 12-deacetyl-12,18-di-*epi*-scalaradial (**4**) (0.5 mg), 12-deacetyl-12-*epi*-scalaradial (**5**) (7.5 mg), 12-deacetyl-12-*epi*-19-deoxyscalarin (**6**) (1.2 mg), and 12-*epi*-scalaradial (**7**) (1.9 mg).

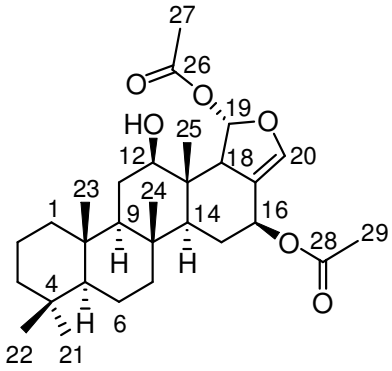
3.2.2 The structure elucidation

3.2.2.1 Heteronemin (3)

Compound **3** was obtained as white needles. The molecular formula of **3** was $C_{29}H_{44}O_6$ according to the pseudomolecular ion peak at *m/z* 511 $[M+Na]^+$ in the ESI-TOF-MS spectrum. The proposed molecular formula required the unsaturation degree of 8, including two carbonyls, one olefin, and five rings. The absorption band in the IR spectrum at 3500 cm^{-1} suggested a hydroxyl group and the band at 1740 and 1235 cm^{-1} did an ester.

The ^1H NMR spectrum (Figure 7) displayed five singlet methyl signals at δ 0.75 (H-22), 0.78 (H-21), 0.80 (H-23), 0.80 (H-24), and 0.86 (H-25). In addition, two acetyl groups were observed at δ 2.06 (H-29) and 2.07 (H-27). A series of olefinic and carbonol protons were observed at δ 6.73 (d; J = 1.2 Hz, H-19), 6.12 (t; J = 2.0 Hz, H-20), 5.32 (ddd; J = 10.5, 6.0, 2.0 Hz, H-16), 3.41 (br d; J = 10.4 Hz,

H-12), and 3.31 (br s; 12-OH). The connection of all the protons was relied on COSY spectral analysis. As for ^{13}C NMR spectrum (Figure 8), 29 carbons were observed. Among these were included two carbonyls, two olefinic carbons, one acetal, and a series of aliphatic carbons (Table 7). The spectrum of **3** was proposed to be heteronemin based on a direct comparison with published data (Kazlauskas *et al*., 1976; Crews and Bascansa, 1986; Wonganuchitmeta *et al*., 2004). The specific rotation ($[\alpha]_D$ -37.6°, MeOH) of **3** was in a comparable range to the report ($[\alpha]_D$ -37°, $CHCl_3$) (Bourquet-Kondracki *et al*., 1994), and indicated that **3** possessed a similar configuration.



3

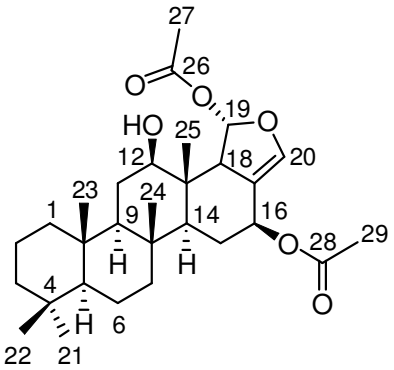


Figure 7 ^{1}H NMR spectrum of **3** ($CDCl_3$; 500 MHz)

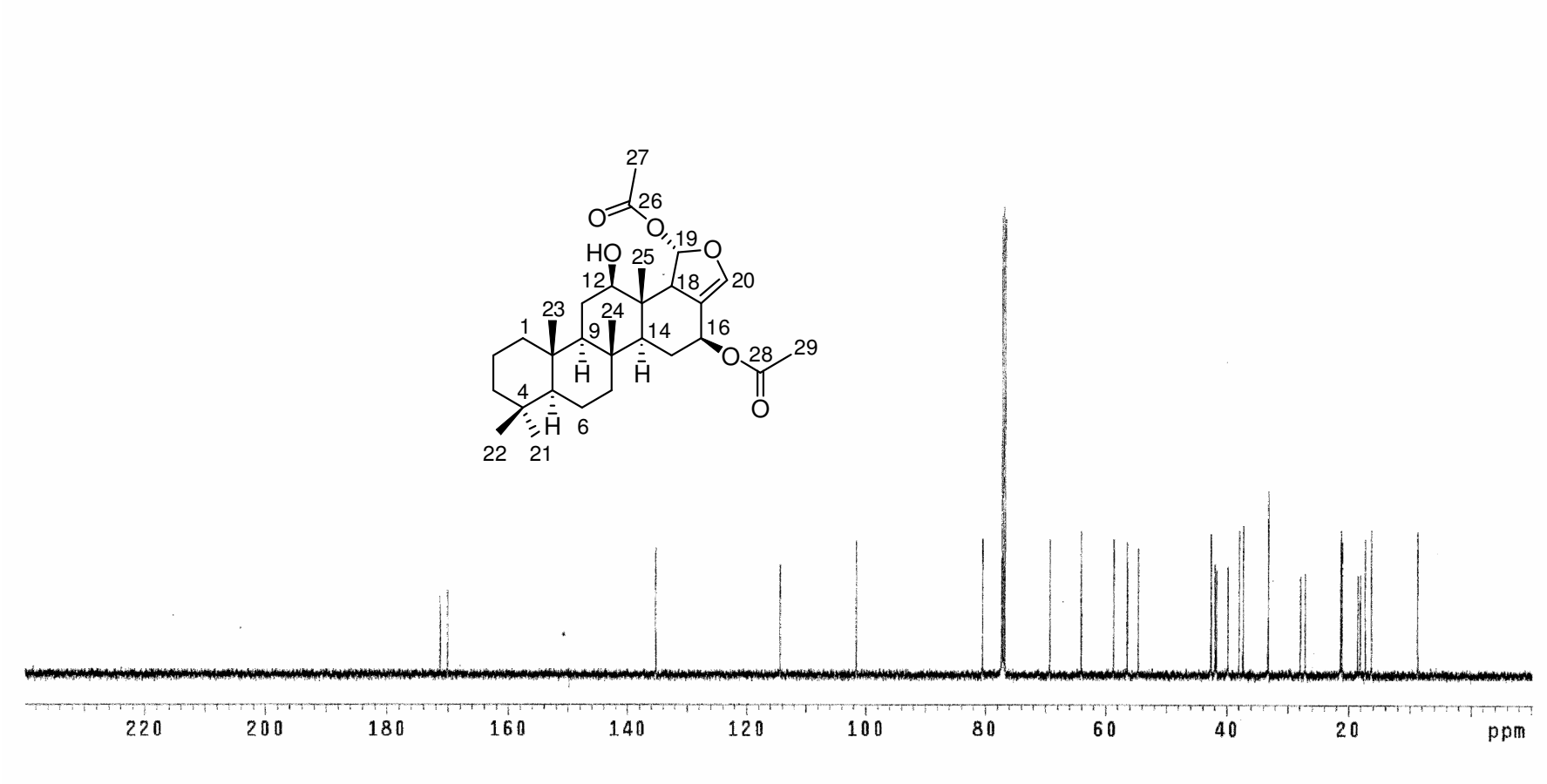


Figure 8 ^{13}C NMR spectrum of **3** ($CDCl_3$; 125 MHz)

Table 7 ^{1}H and ^{13}C NMR spectral data of **3** (500 MHz for ^{1}H; $CDCl_3$)

Position	**^{13}C (mult)**	**^{1}H (mult; *J* in Hz)**
1	39.8 (CH_2)	1.63 (overlap)
		1.67 (overlap)
2	18.1 (CH_2)	1.31 (m)
		1.50 (dd; 13.8, 2.2)
3	42.0 (CH_2)	0.86 (m)
		1.08 (td; 13.6, 4.0)
4	33.2 (C)	-
5	56.4 (CH)	0.73 (m)
6	18.5 (CH_2)	1.42 (m)
		1.57 (m)
7	41.7 (CH_2)	1.32 (m)
		1.69 (m)
8	37.3 (C)	-
9	58.7 (CH)	0.82 (m)
10	38.0 (C)	-
11	27.1 (CH_2)	1.41 (m)
		1.64 (br s)
12	80.5 (CH)	3.41 (br d; 10.4)
12-OH	-	3.31 (br s)
13	42.6 (C)	-
14	54.6(CH)	0.89 (dd; 10.6, 2.2)
15	27.9 (CH_2)	1.37 (m)
		2.02 (ddd; 12.1, 6.0, 2.2)
16	69.3 (CH)	5.32 (ddd; 10.5, 6.0, 2.0)
17	114.4 (C)	-
18	64.1 (CH)	2.39 (br s)
19	101.6 (CH)	6.73 (d; 1.2)
20	135.3 (CH)	6.12 (t; 2.0)

Table 7 (cont.)

Position	^{13}C (mult)	^{1}H (mult; *J* in Hz)
21	33.2 (CH_3)	0.78 (s; 3H)
22	21.3 (CH_3)	0.75 (s; 3H)
23	16.3 (CH_3)	0.80 (s; 3H)
24	17.3 (CH_3)	0.80 (s; 3H)
25	8.7 (CH_3)	0.86 (s; 3H)
26	171.3 (C)	-
27	21.2 (CH_3)	2.07 (s; 3H)
28	170.6 (C)	-
29	21.0 (CH_3)	2.06 (s; 3H)

3.2.2.2 12-Deacetyl-12,18-di-*epi*-scalaradial (4)

Compound **4** was obtained as a white solid (0.5 mg). The molecular formula of $C_{25}H_{38}O_3$ was determined by analysis of the EI mass spectrum, which showed a molecular ion peak at *m/z* 386 $[M]^+$. The degree of unsaturation of 7 was determined as two carbonyls, one olefin and four rings. The major absorption band in the IR spectrum at 3410 cm^{-1} was assigned to the -OH stretching, whereas those at 1720 and 1680 cm^{-1} were the aldehyde functionalities.

The ^{1}H NMR spectra (Figure 9) of **4** differed from compound **3** by the lack of two acetate groups and furan moiety. On the other hand, two aldehyde signals at δ 9.86 (d; *J* = 2.4 Hz, H-19) and 9.41 (s, H-20) were observed. The ^{13}C NMR spectrum (Figure 10) showed corresponding aldehyde carbons at δ 203.0 (C-19) and 193.0 (C-20) (Table 8). The comparison with published data indicated that **4** is 12-deacetyl-12,18-di-*epi*-scalaradial (Walker *et al*., 1980).

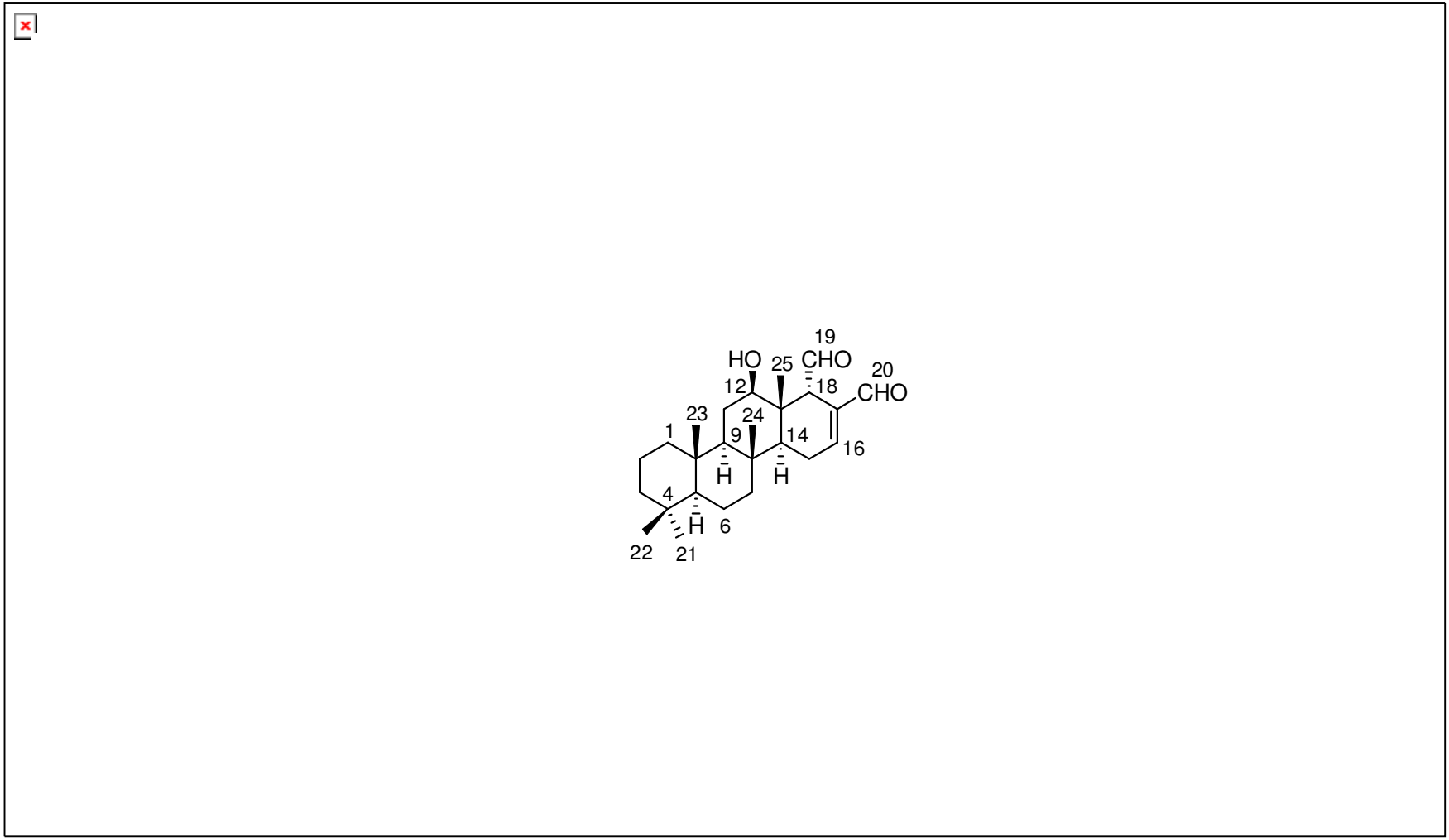


Figure 9 ^{1}H NMR spectrum of **4** ($CDCl_3$; 500 MHz)

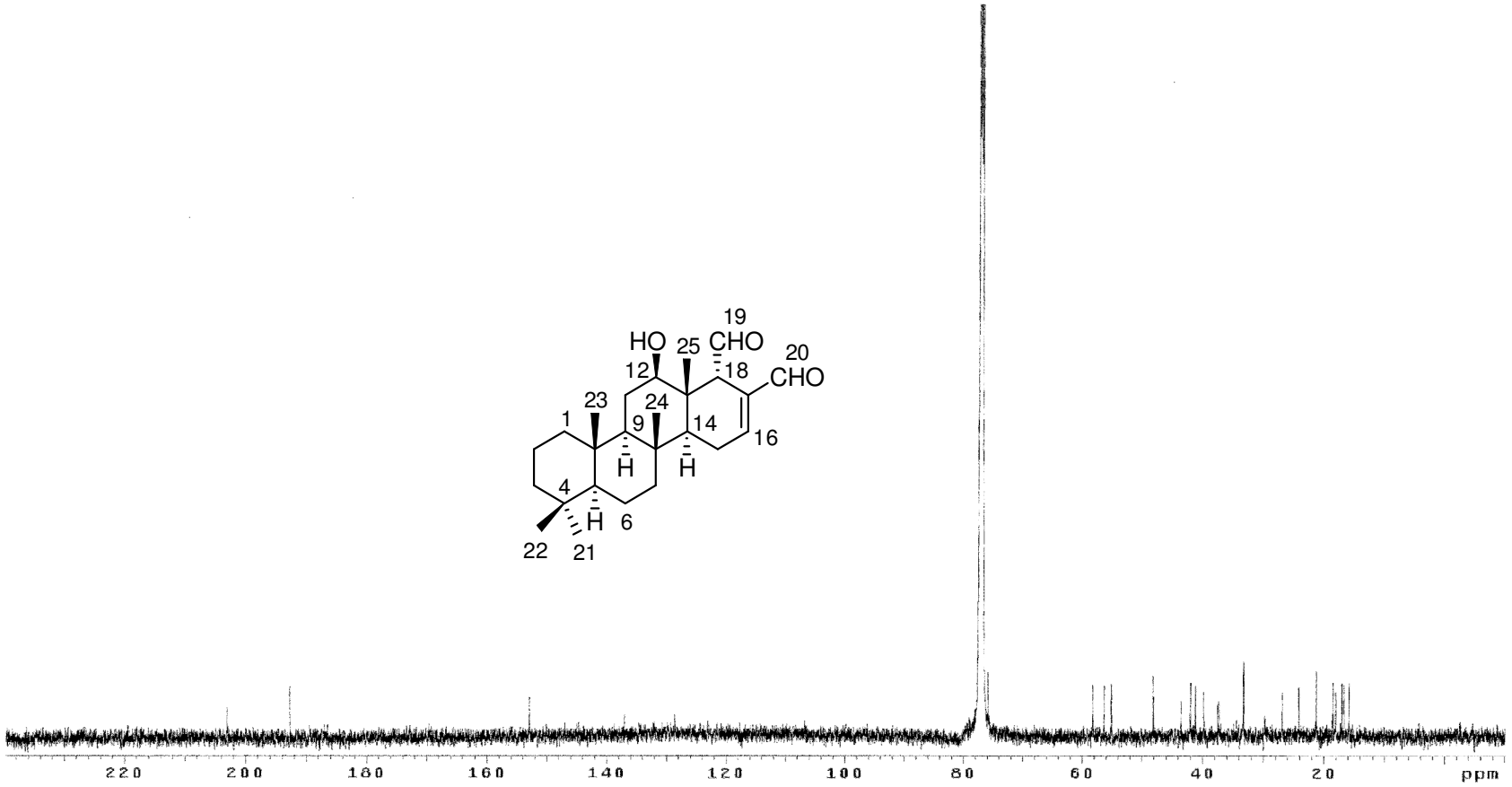
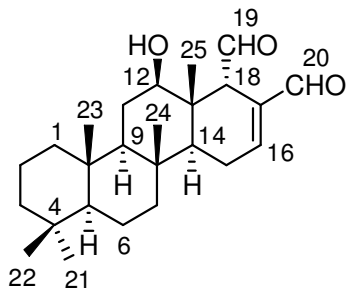


Figure 10 ^{13}C NMR spectrum of **4** ($CDCl_3$; 125 MHz)



4

Table 8 ^{1}H and ^{13}C NMR spectral data of **4** (500 MHz for ^{1}H; $CDCl_3$)

Position	**^{13}C (mult)**	**^{1}H (mult; *J* in Hz)**
1	39.8 (CH_2)	0.78 (m)
		1.63 (m)
2	18.5 (CH_2)	1.10 (td; 13.7, 4.2)
		1.34 (m)
3	42.0 (CH_2)	1.48 (m)
		1.68 (dd; 12.5, 2.7)
4	33.2 (C)	-
5	56.3 (CH)	0.75 (m)
6	18.5 (CH_2)	1.51 (m)
		1.59 (m)
7	41.1 (CH_2)	2.04 (m)
		2.09 (d; 1.5)
8	37.3 (C)	-
9	58.2 (CH)	0.80 (m)
10	37.5 (C)	-
11	26.9 (CH_2)	1.41 (m)
		1.53 (m)
12	75.8 (CH)	3.59 (br d; 10.3)
13	43.5 (C)	-
14	48.1 (CH)	1.39 (dd; 11.5, 5.1)

Table 8 (cont.)

Position	^{13}C (mult)	^{1}H (mult; *J* in Hz)
15	24.2 (CH_2)	2.28 (dd; 20.5, 11.5)
		2.48 (dt; 20.7, 5.1)
16	152.9 (CH)	7.07 (dt; 4.6, 2.7)
17	137.1 (C)	-
18	55.1 (CH)	3.66 (br s)
19	203.0 (CH)	9.86 (d; 2.4)
20	193.0 (CH)	9.41 (s)
21	21.3 (CH_3)	0.78 (s; 3H)
22	18.0 (CH_3)	0.82 (s; 3H)
23	17.0 (CH_3)	0.81 (s; 3H)
24	16.7 (CH_3)	0.89 (s; 3H)
25	15.8 (CH_3)	0.89 (s; 3H)

3.2.2.3 12-Deacetyl-12-*epi*-scalaradial (5)

Compound **5** was isolated as a white solid (7.5 mg). The molecular formula of **5** was deduced to be $C_{25}H_{38}O_3$, from its molecular ion peak at *m/z* 386 $[M]^+$ from the EI mass spectrum. The degree of unsaturation of 7 is assigned for two carbonyls, one olefin and four rings. Similar IR absorptions to those of **4** for the hydroxyl and aldehyde functionalities were also observed.

The ^{1}H (Figure 12) and ^{13}C NMR (Figure 13) spectra of **5** (Table 9) were almost identical to those of **4**. The major differences were that of H-18 (δ 3.11, br s), H-15 (δ 2.34, m), and H-12 (δ 3.71, dd; *J* = 11.5, 4.4 Hz), indicating that **4** and **5** were epimers. A series of nOe-ds experiments, from which the enhancement at δ 3.11 (H-18) and at 0.88 (H-9) due to the irradiation at δ 3.71 (H-12) were observed,

suggested all in the same α-plane (Figure 11). The structure of **5** was confirmed by comparison with the published report (Crews and Bescansa, 1986) to be 12-deacetyl-12-*epi*-scalaradial.

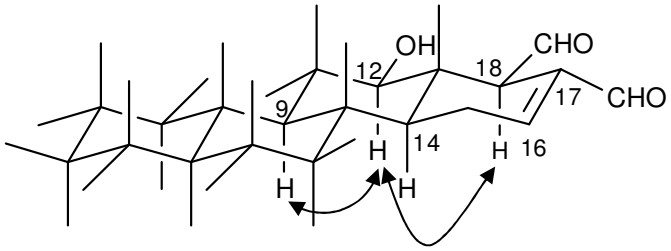
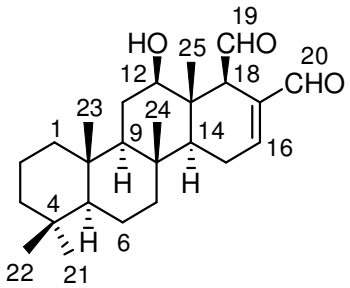


Figure 11 Selected nOe correlations of **5**



5

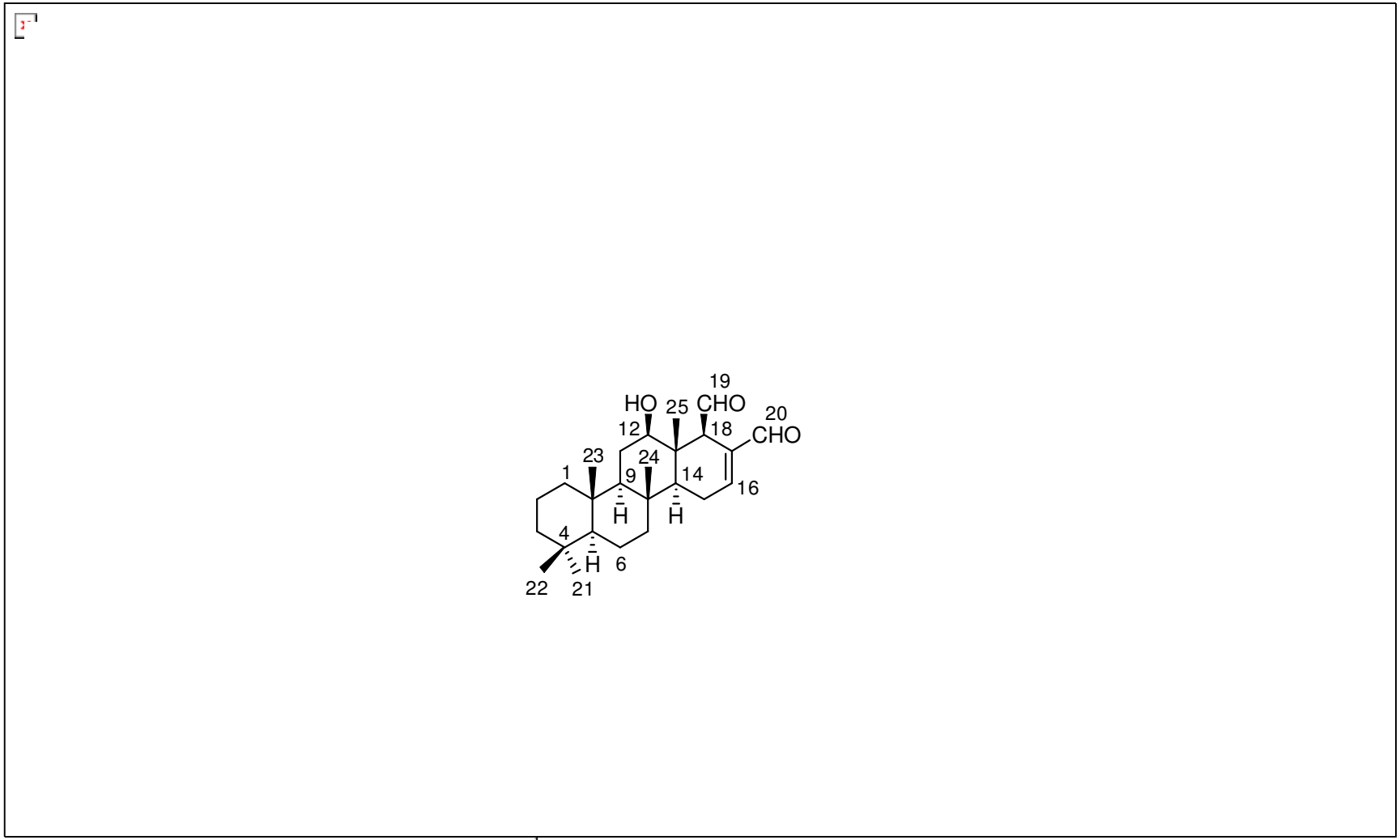


Figure 12 ^{1}H NMR spectrum of **5** ($CDCl_3$; 500 MHz)

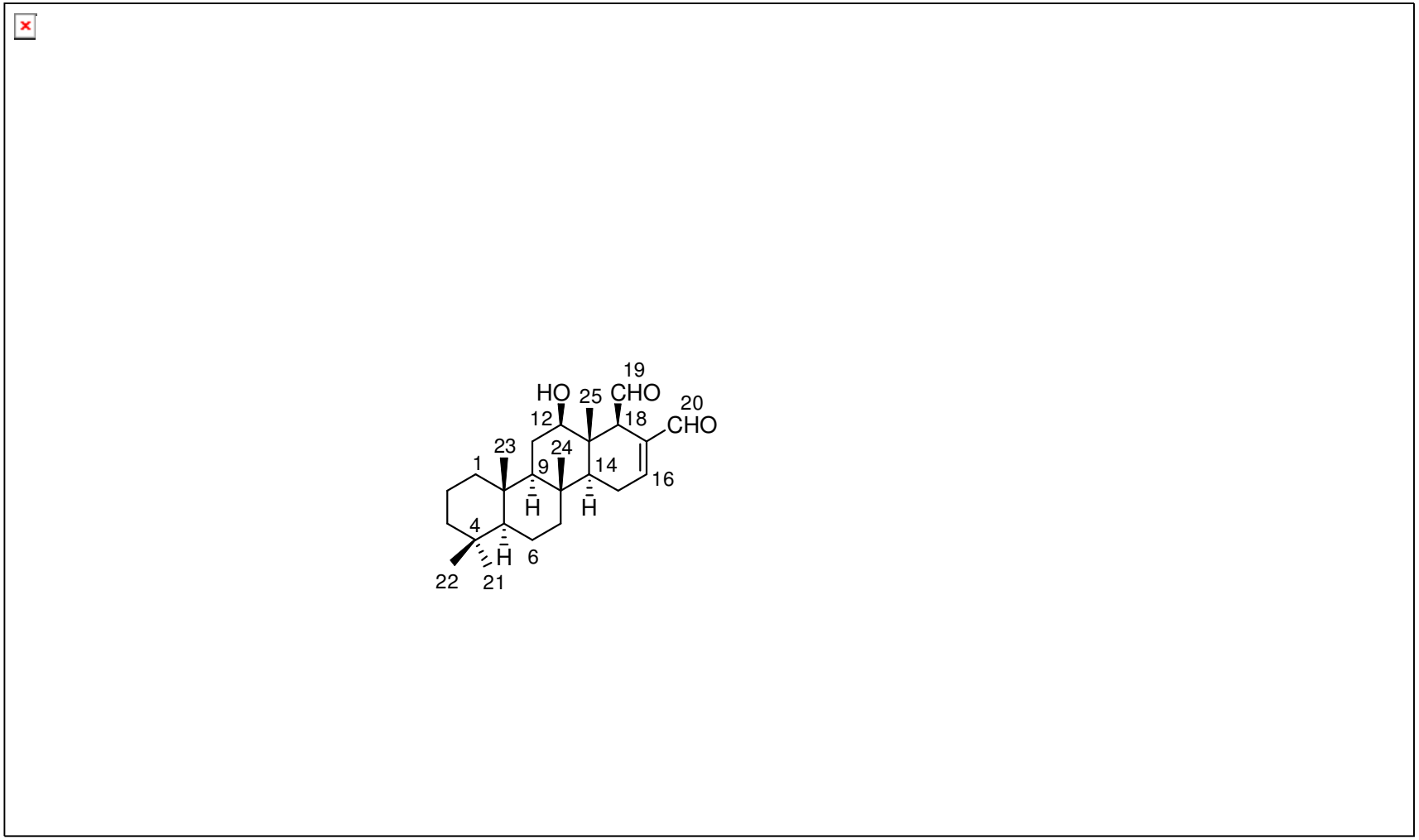


Figure 13 ^{13}C NMR spectrum of **5** ($CDCl_3$; 125 MHz)

Table 9 ^{1}H and ^{13}C NMR spectral data of **5** (500 MHz for ^{1}H; $CDCl_3$)

Position	**^{13}C (mult)**	**^{1}H (mult; *J* in Hz)**
1	39.8 (CH_2)	0.77 (m)
		1.63 (m)
2	18.5 (CH_2)	1.36 (m)
		1.40 (m)
3	42.0 (CH_2)	1.33 (m)
		1.68 (br d; 12.2)
4	33.2 (C)	-
5	56.4 (CH)	0.76 (d; 2.4)
6	18.0 (CH_2)	1.53 (m)
		1.59 (dt; 13.4, 3.4)
7	41.5 (CH_2)	0.91 (m)
		1.73 (dt; 12.7, 3.4)
8	37.3 (C)	-
9	58.1 (CH)	0.88 (m)
10	37.6 (C)	-
11	27.1 (CH_2)	1.41 (dd; 11.7, 4.9)
		1.56 (m)
12	81.0 (CH)	3.71 (dd; 11.5, 4.4)
13	43.8 (C)	-
14	53.1 (CH)	1.11 (dt; 13.4, 4.2)
15	23.8 (CH_2)	2.34 (m)
		2.44 (m)
16	153.4 (CH)	7.08 (dt; 4.9, 2.4)
17	138.9 (C)	-
18	60.4 (CH)	3.11 (br s)
19	204.5 (CH)	9.76 (d; 3.7)
20	192.9 (CH)	9.39 (s)

Table 9 (cont.)

Position	^{13}C (mult)	^{1}H (mult; *J* in Hz)
21	17.1 (CH_3)	0.93 (s; 3H)
22	21.3 (CH_3)	0.87 (s; 3H)
23	33.2 (CH_3)	0.82 (s; 3H)
24	16.5 (CH_3)	0.82 (s; 3H)
25	9.9 (CH_3)	0.78 (s; 3H)

3.2.2.4 12-Deacetyl-12-*epi*-19-deoxyscalarin (6)

Compound **6** was obtained as a white solid (1.2 mg). The EI mass spectrum showed a molecular ion peak at *m/z* 386 $[M]^+$, indicating a molecular formula of $C_{25}H_{38}O_3$. The proposed molecular formula requires the unsaturation degrees of 7, i.e., one carbonyl, one double bond and five rings. The IR absorption band at 3420 cm^{-1} suggested a hydroxyl group and at 1740 cm^{-1} did an ester functionality.

Whereas the molecular formulae of **4**, **5**, and **6** were similar, **6** was devoided of the aldehydes and instead possessed a lactone moiety (δ 170.0, C-20) that was derived from the two aldehyde carbons previously observed in **4** and **5**, all resided on the same scalarane skeleton (Figure 14 and 15, and Table 10). Compound **6** was identified as 12-deacetyl-12-*epi*-19-deoxyscalarin based on the comparison with the reported data (Cimino *et al*., 1977).

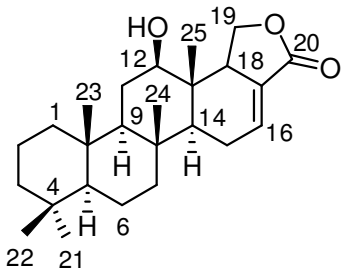


Figure 14 ^{1}H NMR spectrum of **6** ($CDCl_3$; 500 MHz)

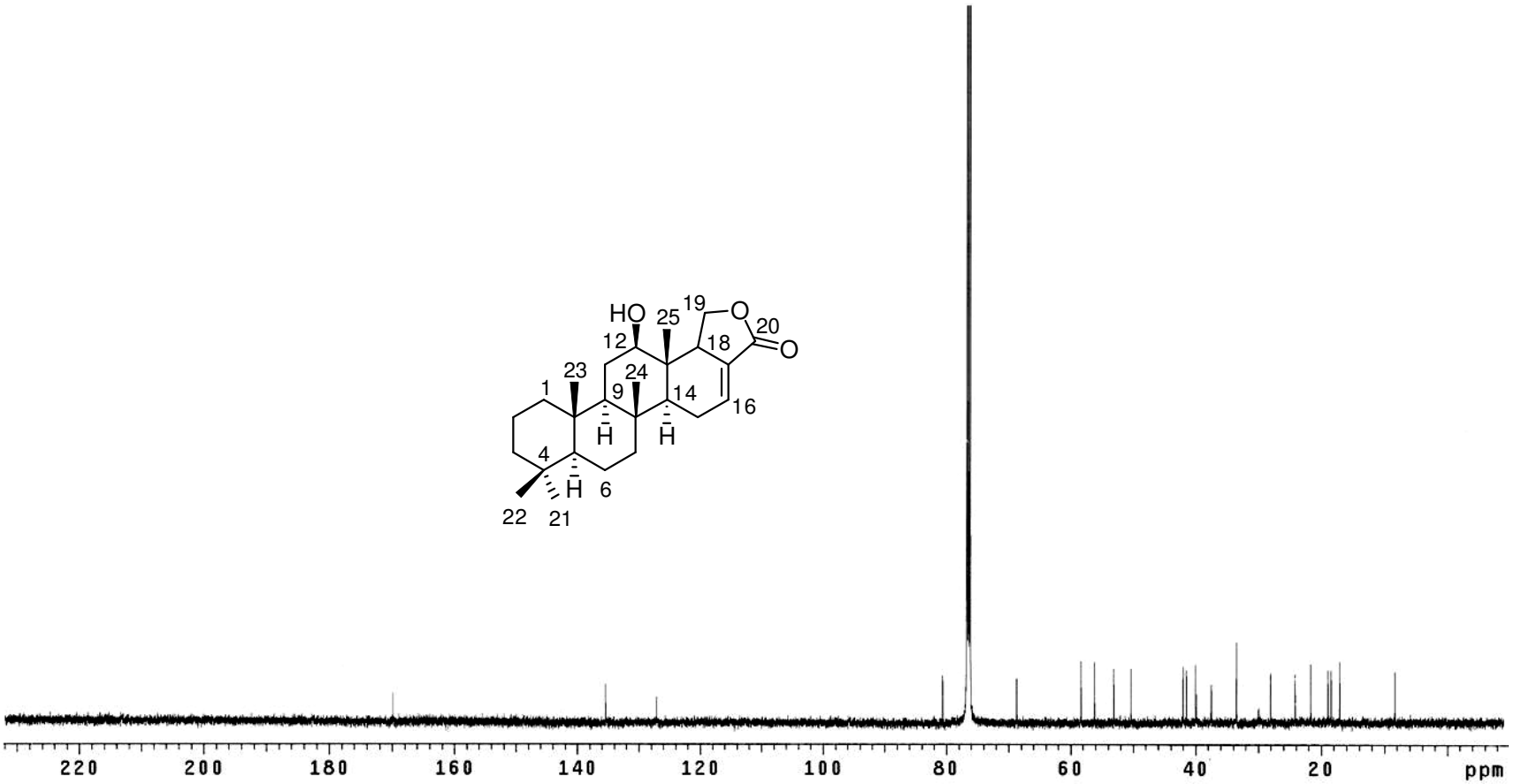
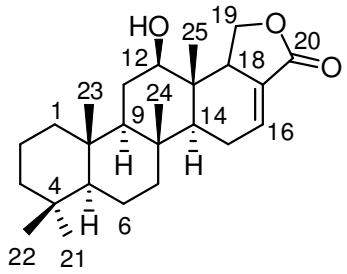


Figure 15 ^{13}C NMR spectrum of **6** ($CDCl_3$; 125 MHz)



6

Table 10 ^{1}H and ^{13}C NMR spectral data of **6** (500 MHz for ^{1}H; $CDCl_3$)

Position	^{13}C (mult)	^{1}H (mult; *J* in Hz)
1	39.9 (CH_2)	0.88 (m)
		1.65 (m)
2	18.5 (CH_2)	1.40 (m)
		1.43 (m)
3	42.0 (CH_2)	1.32 (m)
		1.68 (m)
4	33.3 (C)	-
5	56.4 (CH)	0.78 (m)
6	18.0 (CH_2)	1.36 (m)
		1.52 (m)
7	41.4 (CH_2)	1.60 (dt; 13.7, 3.4)
		1.70 (dt; 13.0, 3.4)
8	37.4 (C)	-
9	58.7 (CH)	0.89 (m)
10	37.4 (C)	-
11	27.7 (CH_2)	1.64 (m)
		1.66 (m)
12	81.2 (CH)	3.41 (dd; 11.2, 4.2)
13	39.8 (C)	-
14	53.3 (CH)	1.23 (m)
15	23.7 (CH_2)	2.12 (m)
		2.32 (m)

Table 10 (cont.)

Position	^{13}C (mult)	^{1}H (mult; *J* in Hz)
16	135.4 (CH)	6.83 (dt; 6.8, 3.4)
17	127.2 (C)	-
18	50.5 (CH)	2.82 (m)
19	69.2 (CH_2)	4.18 (t; 9.6)
		4.49 (t; 9.5)
20	170.0 (C)	-
21	21.3 (CH_3)	0.79 (s; 3H)
22	33.3 (CH_3)	0.83 (s; 3H)
23	16.6 (CH_3)	0.84 (s; 3H)
24	16.6 (CH_3)	0.91 (s; 3H)
25	7.8 (CH_3)	0.74 (s; 3H)

3.2.2.5 12-*epi*-Scalaradial (7)

Compound **7** was obtained as a white solid (1.9 mg). The EI mass spectrum of **7** showed a molecular ion peak at *m/z* 428 $[M]^+$, indicating the molecular formula of $C_{27}H_{40}O_4$. The eight-degree of unsaturation is assigned as three carbonyls, one olefin and four ring systems. The IR spectrum showed absorption band at 1740 cm^{-1} suggested aldehyde functionality.

Compared with the ^{1}H and ^{13}C NMR spectra of **4** and **5**, **7** (Figure 16 and 17) was also almost identical to both. The missing hydroxyl absorption band in the IR spectrum, and the additional acetate moiety (δ_C 170.0, C-26; 21.2, C-27) suggested the **7** was the acetate derivatives of **5** (Table 11). This was confirmed by the comparison with previous reported (Cimino *et al.*, 1977). Compound **7** was therefore identified as 12-*epi*-scalaradial.

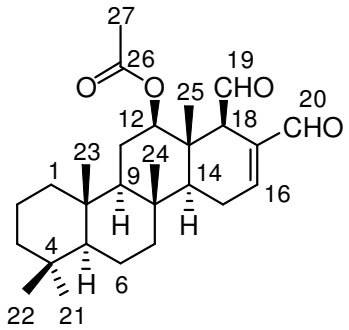


Figure 16 ^{1}H NMR spectrum of **7** ($CDCl_3$; 500 MHz)

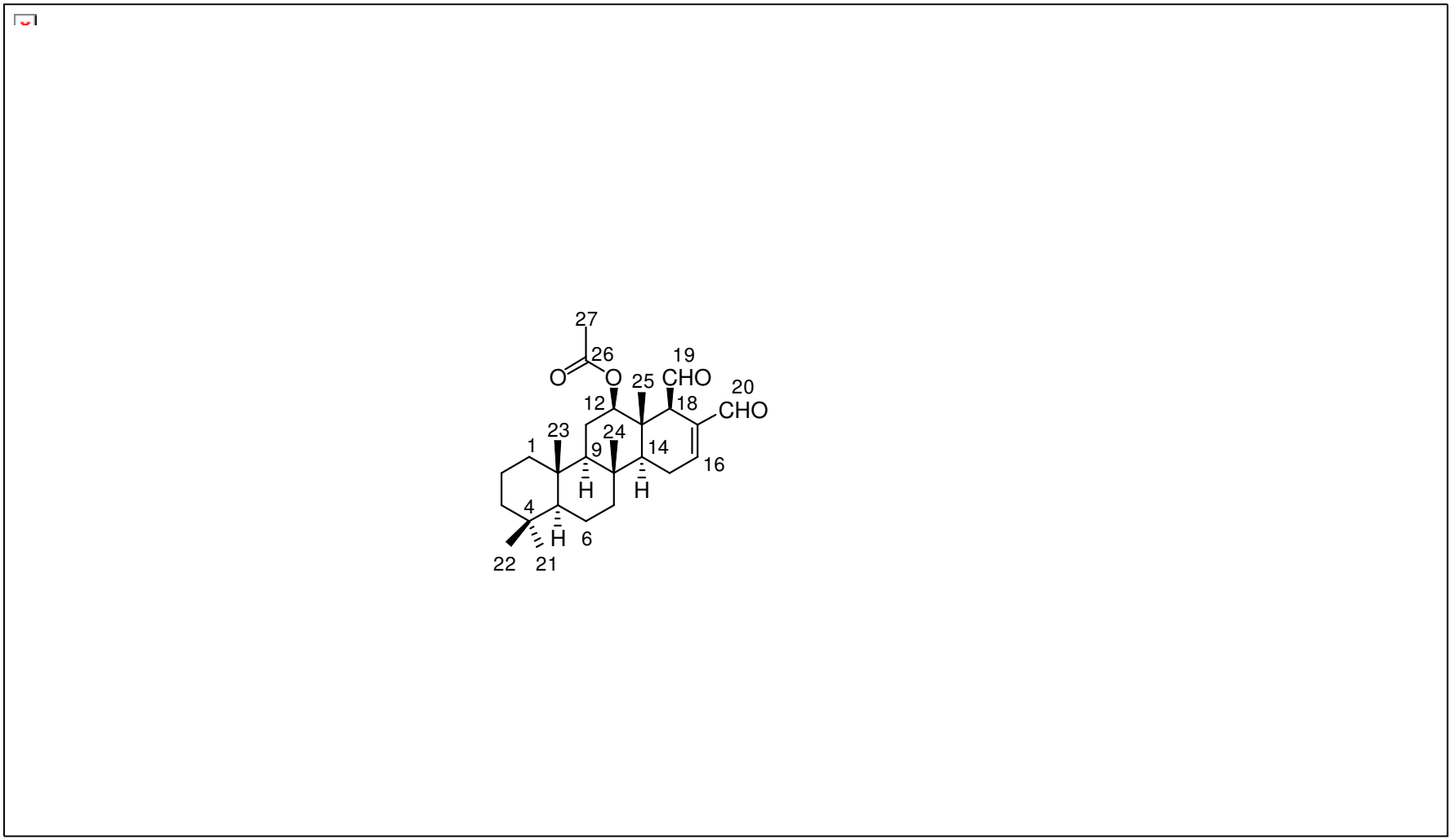
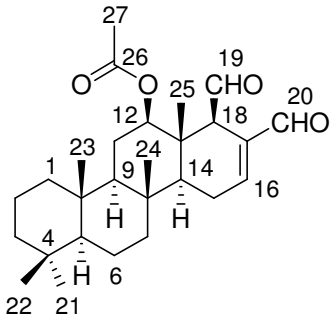


Figure 17 ^{13}C NMR spectrum of **7** ($CDCl_3$; 125 MHz)



7

Table 11 ^{1}H and ^{13}C NMR spectral data of **7** (500 MHz for ^{1}H; $CDCl_3$)

Position	**^{13}C (mult)**	**^{1}H (mult; *J* in Hz)**
1	39.6 (CH_2)	0.84 (m)
		1.62 (dt; 13.9, 2.7)
2	18.3 (CH_2)	1.34 (m)
		1.55 (m)
3	41.4 (CH_2)	0.90 (m)
		1.33 (m)
4	33.2 (C)	-
5	56.4 (CH)	0.78 (m)
6	18.0 (CH_2)	1.42 (m)
		1.51 (m)
7	41.9 (CH_2)	0.86 (m)
		1.74 (dt; 12.4, 3.2)
8	37.3 (C)	-
9	57.7 (CH)	0.98 (m)
10	37.6 (C)	-
11	23.6 (CH_2)	2.38 (m)
		2.40 (m)
12	82.1 (CH)	4.79 (dd; 11.5, 4.4)
13	42.0 (C)	-
14	53.4 (CH)	1.23 (m)

Table 11 (cont.)

Position	^{13}C (mult)	^{1}H (mult; *J* in Hz)
15	23.5 (CH_2)	1.38 (m)
		1.86(ddd; 12.7, 4.2, 1.5)
16	152.8 (CH)	7.03 (m)
17	139.5 (C)	-
18	60.0 (CH)	3.10 (br s)
19	200.0 (CH)	9.68 (d; 3.7)
20	192.1 (CH)	9.34 (s)
21	33.2 (CH_3)	0.82 (s; 3H)
22	21.4 (CH_3)	0.78 (s; 3H)
23	17.1 (CH_3)	0.96 (s; 3H)
24	16.5 (CH_3)	0.81 (s; 3H)
25	11.0 (CH_3)	0.97 (s; 3H)
26	170.0 (C)	-
27	21.2 (CH_3)	2.01 (s; 3H)

3.3 Biological activities of the isolated compounds

All the isolated compounds from *Aplysinopsis* sp. were investigated for antitubercular activity against *M. tuberculosis* (Changsen *et al*., 2003). The results were showed in Table 12. Compound **3** was also investigated for antimalarial activity against *P. falciparum*, cytotoxic activity against cancer cell line (MCF-7), and antimicrobial activity using *B. subtilis*, *S. aureus*, *E. coli*, *T. rubrum*, and *C. albicans* as microorganism targets (Lorian, 1996; Salie *et al*., 1996).

Table 12 Bioactivity of isolated compounds from the sponge *Aplysinopsis* sp.

Compounds	**Anti-TB (MIC; μM)**
heteronemin (**3**)	6
12-deacetyl-12,18-di-*epi*-scalaradial (**4**)	32
12-deacetyl-12-*epi*-scalaradial (**5**)	32
12-deacetyl-12-*epi*-19-deoxyscalarin (**6**)	64
12-*epi*-scalaradial (**7**)	7
rifampicin	0.0036 - 0.0145
streptomycin	0.2682 - 0.5380
isoniazid	0.1677 - 0.3354
ofloxacin	1.0819 - 2.1612

All the isolated compound exhibited antitubercular activity against *M. tuberculosis* with the MICs in the range from 6 to 64 μM. Compounds **3** also showed antimalarial activity against *P. falciparum* with an IC_{50} of 9 μM and cytotoxic activity against MCF-7 cell line with an IC_{50} of 0.12 μM. However, **3** did not showed any antimicrobial activity against all other bacteria and fungi targeted in this investigation at the highest concentration of 500 μg/disc.

CHAPTER 4

CONCLUSION

In this study, the investigation for the chemical constituents *Halichondria* sp. led to the isolation of a mixture of two steroids, dihydrocholesterol (**1**) and (3β, 24S)-stigmast-5-en-3-ol (**2**), and the investigation for *Aplysinopsis* sp. led to the isolation of five members of the scalarane-type sesterterpenes including, heteronemin (**3**), 12-Deacetyl-12,18-di-*epi*-scalaradial (**4**), 12-Deacetyl-12-*epi*-scalaradial (**5**), 12-Deacetyl-12-*epi*-19-deoxyscalarin (**6**) and 12-*epi*-Scalaradial (**7**).

From the results of bioactivity evaluation heteronemin (**3**) showed antimalarial activity against *Plasmodium falciparum* K1 strain (IC_{50} 9 μM) and showed cytotoxicity (against MCF-7 cell line) assay (IC_{50} 0.12 μM). Compounds **3**, **4**, **5**, **6**, and **7** exhibited potent antitubercular activity against *Mycobacterium tuberculosis* H_{37}Ra (MICs 6, 32, 32, 64, and 7 μM, respectively).

REFERENCES

Blunt, J.W., Copp, B.R., Munro, M.H., Northcote, P.T. and Prinsep, M.R. 2005. Marine natural products. *Nat. Prod. Rep.* 22: 15-61.

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

Capon, R.J., Ghisalberti, E.L. and Jefferies, P.R. 1982. New aromatic sesquiterpenes from a *Halichondria* sp. *Aust. J. Chem.* 35: 2583-2587.

Changsen, C., Franzblau, S.G. and Palittapongarnpim, P. 2003. Improved green fluorescent protein reporter gene-based microplate screening for antituberculosis compounds by utilizing an acetamidase promoter. *Antimicrob. Agents Chemother.* 47: 3682-3687.

Chill, L., Yosief, T. and Kashman, Y. 2002. Halichondramine, a new tetracyclic bipiperidine alkaloid from the marine sponge *Halichondria* sp. *J. Nat. Prod.* 65: 1738-1741.

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

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

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

Desjadins, R.E., Canfield, C.J., Haynes, J.D. and Chulay, J.D. 1979. Quantitative assessment of antimalarial activity *in vitro* by a semiautomated microdilution technique. *Antimicrob. Agents Chemother*. 16: 710-718.

Erpenbeck, D. and van Soest, R.W.M. 2005. A survey for biochemical synapomorphies to reveal phylogenetic relationships of halichondrid demosponges (Metazoa: Porifera). *Biochem. Syst. Ecol.* 33: 585-616.

Faulkner, D.J. 2002. Marine natural products. *Nat. Prod. Rep.* 19: 1-48.

Faulkner, D.J., Newman, D.J. and Cragg, G.M. 2004. Investigations of the marine flora and fauna of the Islands of Palau. *Nat. Prod. Rep.* 21: 50-76.

Fusetani, N., Matsunaga, S. and Konosu, S. 1981. Bioactive marine metabolites II. Halistanol sulfate, an antimicrobial novel steroid sulfate from the marine sponge *Halichondria* cf. *moorei* Bergquist. *Tetrahedron Lett.* 22(21): 1985-1988.

Garson, M.J. and Simpson, J.S. 2004. Marine isocyanides and related natural products structure, biosynthesis and ecology. *Nat. Prod. Rep.* 21: 164-179.

Hirata, Y. and Uemura, D. 1986. Halichondrins-antitumor polyether macrolides from a marine sponge. *Pure Appl. Chem.* 58(5): 701-710.

Ishiyama, H., Hashimoto, A., Fromont, J., Hoshino, Y., Mikami, Y. and Kobayashi, J. 2005. Halichonadins A-D, new sesquiterpenoids from a sponge *Halichondria* sp. *Tetrahedron* 61: 1101-1105.

Ishiyama, H., Kozawa, S., Aoyama, K., Mikami, Y., Fromont, J. and Kobayashi, J. 2008. Halichonadin F and the Cu(I) complex of halichonadin C from the sponge *Halichondria* sp. *J. Nat. Prod.* 71: 1301-1303.

Jimenez, C., Quinoa, E., Adamczeski, M., Hunter, L.M. and Crews, P. 1991. Novel sponge-derived amino acids. 12. Tryptophan-derived pigments and accompanying sesterterpenes from *Fascaplysinopsis reticulata*. *J. Org. Chem.* 56: 3403-3410.

Jongrungruangchok, S., Kittakoop, P., Yongsmith, B., Bavovada, R., Tanasupawat, S., Lartpornmatulee, N. and Thebtaranonth, Y. 2004. Azaphilone pigments from a yellow mutant of the fungus *Monascus kaoliang*. *Phytochem. Anal.* 65: 2569-2575.

Kanazawa, S., Fusetani, N. and Matsunaga, S. 1993. Cylindramide: Cytotoxic tetramic acid lactam from the marine sponge *Halichondria cylindrata* Tanita & Hoshino. *Tetrahedron Lett.* 34(6): 1065-1068.

Kassuhlke, E.K., Potts, B.C.M. and Faulkner, D.J. 1991. New nitrogenous sesquiterpenes from two Philippine nudibranchs, *Phyllidia pustulosa* and *P. varicose*, and from a Palauan sponge, *Halichondria* cf. *lendenfeldi*. *J. Org. Chem.* 56: 3747-3750.

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

Kernan, M.R. and Faulkner, D.J. 1987. Halichondramine, an antifungal macrolide from the sponge *Halichondria* sp. *Tetrahedron Lett.* 28(25): 2809-2812.

Kernan, M.R., Molinski, T.F. and Faulkner, D.J. 1988. Macrocyclic antifungal metabolites from the Spanish dancer nudibranch *Hexabranchus sanguineus* and sponge of the genus *Halichondria*. *J. Org. Chem.* 53: 5014-5020.

Kobayashi, J., Tsuda, M., Fuse, H., Sasaki, T. and Mikami, Y. 1997. Halishigamides A-D, new cytotoxic oxazole-containing metabolites from Okinawan sponge *Halichondria* sp. *J. Nat. Prod.* 60: 150-154.

Kozawa, S., Ishiyama, H., Fromont, J. and Kobayashi, J. 2008. Halichonadin E, a dimeric sesquiterpenoid from the sponge *Halichondria* sp. *J. Nat. Prod.* 71: 445-447.

Kuramoto, M., Tong, C., Yamada, K., Chiba, T., Hayashi, Y. and Uemura, D. 1996. Halichlorine, an inhibitor of VCAM-1 induction from the marine sponge *Halichondria okadai* Kadota. *Tetrahedron Lett.* 37(22): 3867-3870.

Li, H.Y., Matsunaga, S. and Fusetani, N. 1995. Halicylindrosides, antifungal and cytotoxic cerebrosides from the marine sponge *Halichondria cylindrata. Tetrahedron* 51(8): 2273-2280.

Li, H.Y., Matsunaga, S. and Fusetani, N. 1995. Halicylindramides A-C, antifungal and cytotoxic depsipeptides from the marine sponge *Halichondria cylindrata. J. Med. Chem.* 38: 338-343.

Li, H.Y., Matsunaga, S. and Fusetani, N. 1996. Halicylindramides D and E, antifungal peptides from the marine sponge *Halichondria cylindrata*. *J. Nat. Prod.* 59(2): 163-166.

Lorian, V. 1996. *Antibiotics in laboratory medicine*. 4th ed. Baltimore: Williams & Wilkins.

Mancini, I., Guella, G., Zibrowius, H. and Pietra, F. 2003. On the origin of quasi-racemic aplysinopsin cycloadducts, (bis)indole alkaloids isolated from scleractinian corals of the family Dendrophylliidae. Involvement of

enantiodefective diels-alderases or asymmetric induction in artifact processes involving adventitious catalysts?. *Tetrahedron* 59: 8757-8762.

Molinski, T.F., Faulkner, D.J., Duyne, G.D. and Clardy, J. 1987. Three new diterpene isonitriles from a palauan sponge of the genus *Halichondria*. *J. Org. Chem.* 52: 3334-3337.

Molinski, T.F., Dalisay, D.S., Lievens, S.L. and Saludes, J.P. 2009. Drug development from marine natural products. *Nat. Rev. Drug Disc.* 8: 69-85.

Nakagawa, M., Hamamoto, Y., Ishihama, M., Hamasaki, S. and Endo, M. 1987. Pharmacologically active homosesterterpenes from Palauan sponges. *Tetrahedron Lett.* 28: 431-434.

Nakamura, H., Deng, S., Takamatsu, M., Kobayashi, J., Ohizumi, Y. and Hirata, Y. 1991. Structure of halipanicine, a new sesquiterpene isothiocyanate from the Okinawan marine sponge *Halichondria panicea* (Pallas). *Agric. Biol. Chem.* 55(2): 581-583.

Niwa, H., Wakamatsu, K. and Yamada, K. 1989. Halicholactone and neohalicholactone, two novel fatty acid metabolites from the marine sponge *Halichondria okadai* Kadota. *Tetrahedron Lett.* 30: 4543-4546.

Osinga, R., Tramper, J. and Wijffels, R.H. 1998. Cultivation of marine sponges for metabolite production: applications for biotechnology. *Trends Biotechnol.* 16: 130-134.

Roll, D.M., Ireland, C.M., Lu, H.S.M. and Clardy, J. 1988. Fascaplysin, an unusual antimicrobial pigment from the marine sponge *Fascaplysinopsis* sp. *J. Org. Chem.* 53: 3276-3278.

Salie, F., Eagles, P.F.K. and Leng, H.M.J. 1996. Preliminary antimicrobial screening of four South African Asteraceae species. *J. Ethnopharmacol.* 52(1): 27-33.

Simmons, T.L. Andrianasolo, E., McPhail, K., Flatt, P. and Gerwick, W.H. 2005. Marine natural products as anticancer drugs. *Mol. Cancer Ther.* 4: 333-342.

Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Warren, J.T., Bokesch, H., Kenney, S. and Boyd, M.R. 1990. New colorimetric cytotoxicity assay for anticancer-drug screening. *J. Natl. Cancer Inst.* 82: 1107-1112.

Sullivan, B.W., Faulkner, D.J., Okamoto, K.T., Chen, M.H.M. and Clardy, J. 1986. (6*R*,7*S*)-7-amino-7,8-dihydro-α-bisabolene, an antimicrobial metabolite from the marine sponge *Halichondria* sp. *J. Org. Chem.* 51: 5134-5136.

Sung, P.J., Chen, W.C., Chen, Y.P., Li, J.J., Fang, L.S. and Sheu, J.H. 2006. Occurrence of isoquinoline quinones in the sponge *Halichondria* sp. (Halichondriidae). *Biochem. Syst. Ecol.* 34: 174-176.

Tachibana, K., Scheuer, P.J., Tsukitani, Y., Kikuchi, H., Engen, D., Clardy, J., Gopichand, Y. and Schmitz, F.J. 1981. Okadaic acid, a cytotoxic polyether from two marine sponges of the genus Halichondria. *J. Am. Chem. Soc.* 103: 2469-2471.

Trager, W. and Jensen, J.B. 1976. Human malaria parasites in continuous culture. *Science* 193: 673-675.

Uemura, D., Takahashi, K., Yamamoto, T., Katayama, C., Tanaka, J., Okumura, Y. and Hirata, Y. 1985. Norhalichondrin A: an antitumor polyether macrolide from a marine sponge. *J. Am. Chem. Soc.* 107: 4796-4798.

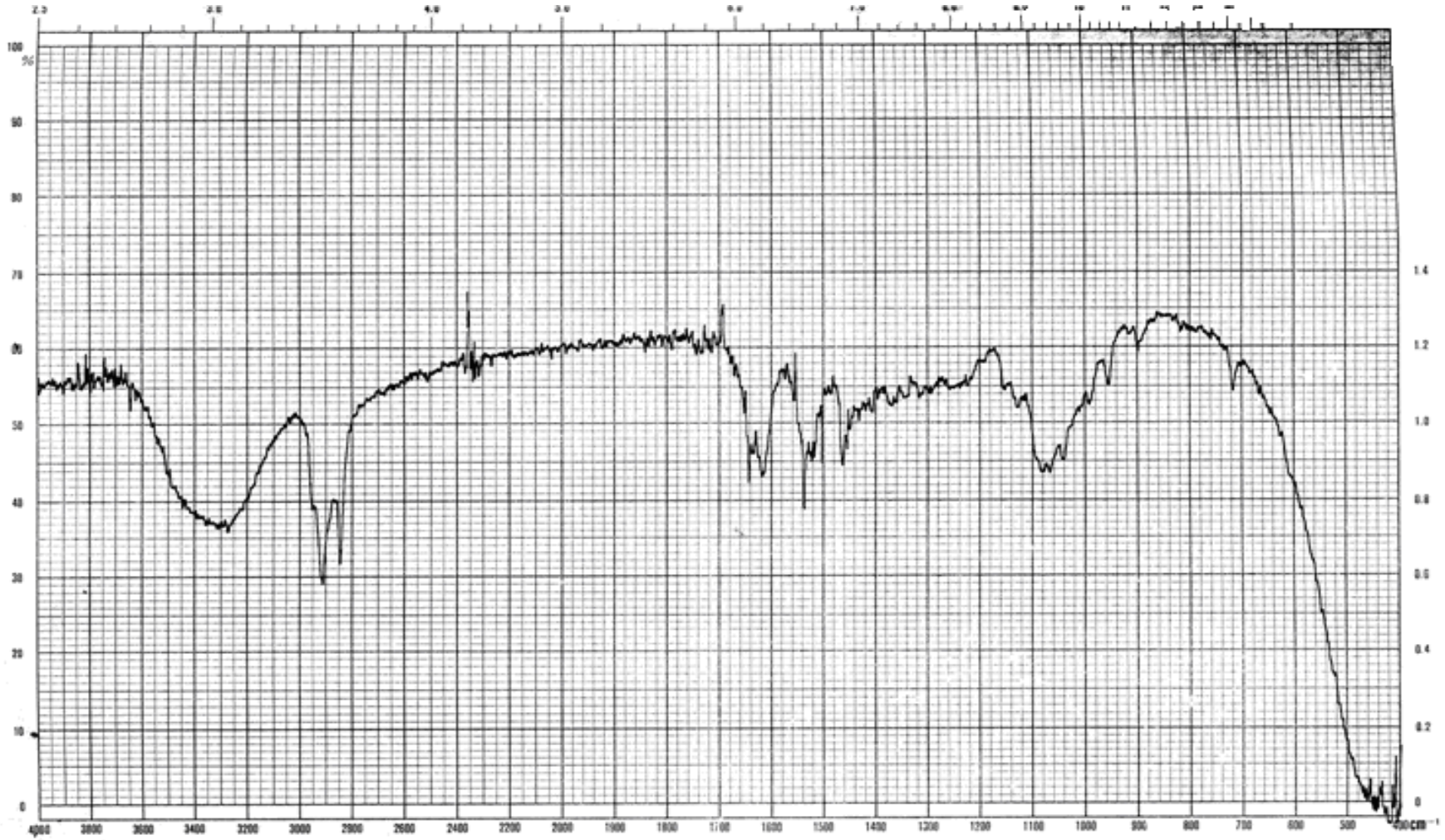
Ueoka, R., Nakao, Y., Fujii, S., van Soest, R.W.M. and Matsunaga, S. 2008. Aplysinoplides A-C, cytotoxic sesterterpenes from the marine sponge *Aplysinopsis digitata*. *J. Nat. Prod.* 71: 1089-1091.

Ungur, N. and Kulcitki, V. 2009. Occurrence, biological activity and synthesis of cheilanthane sesterterpenoids. *Tetrahedron* 65: 3815-3828.

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

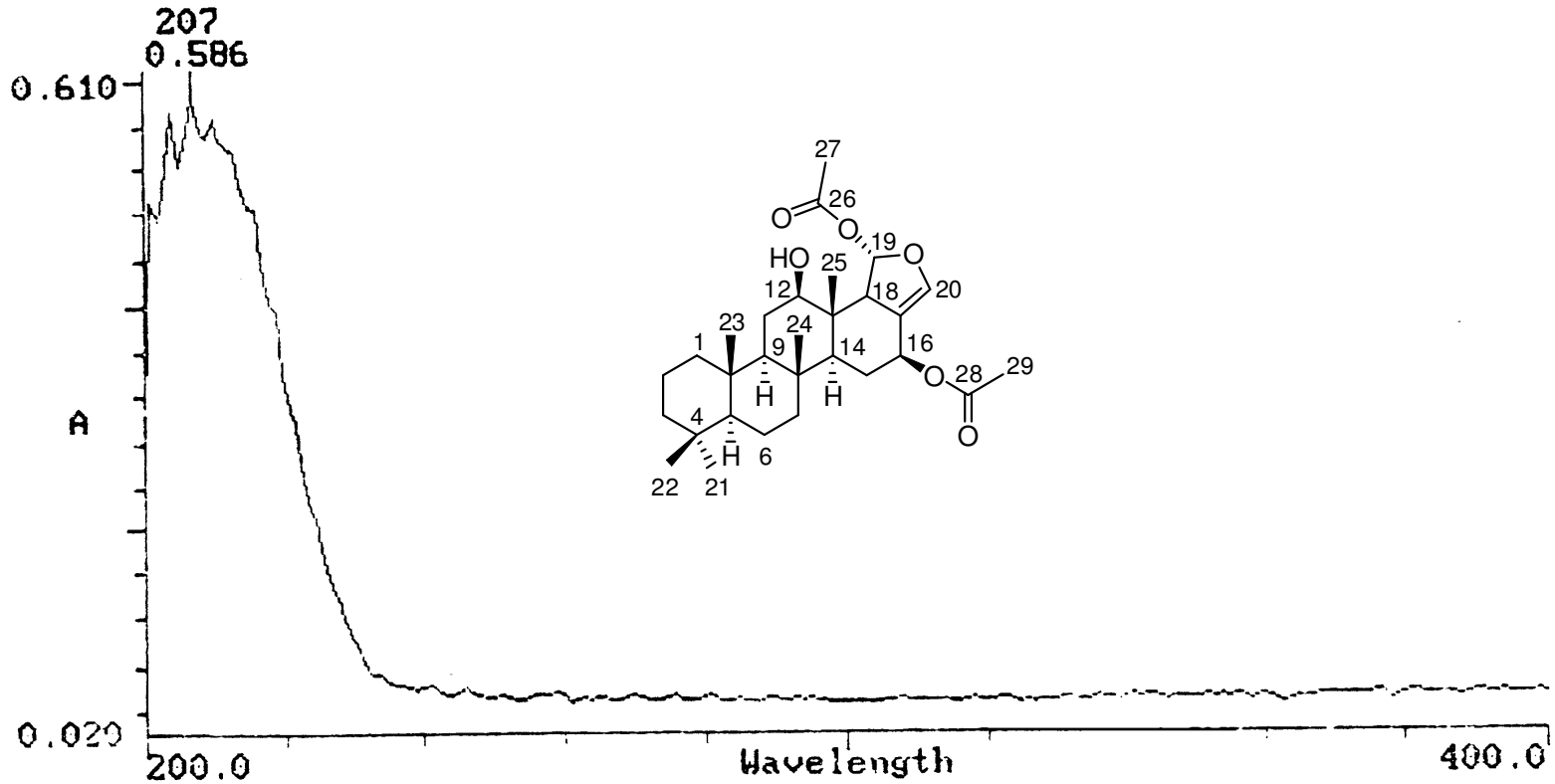
Wonganuchitmeta, S., Yuentongsawad, S., Keawpradub, N. and Plubrukarn, A. 2004. Antitubercular sesterterpenes from the Thai sponge *Brachiaster* sp. *J. Nat. Prod.* 67: 1767-1770.

APPENDIX

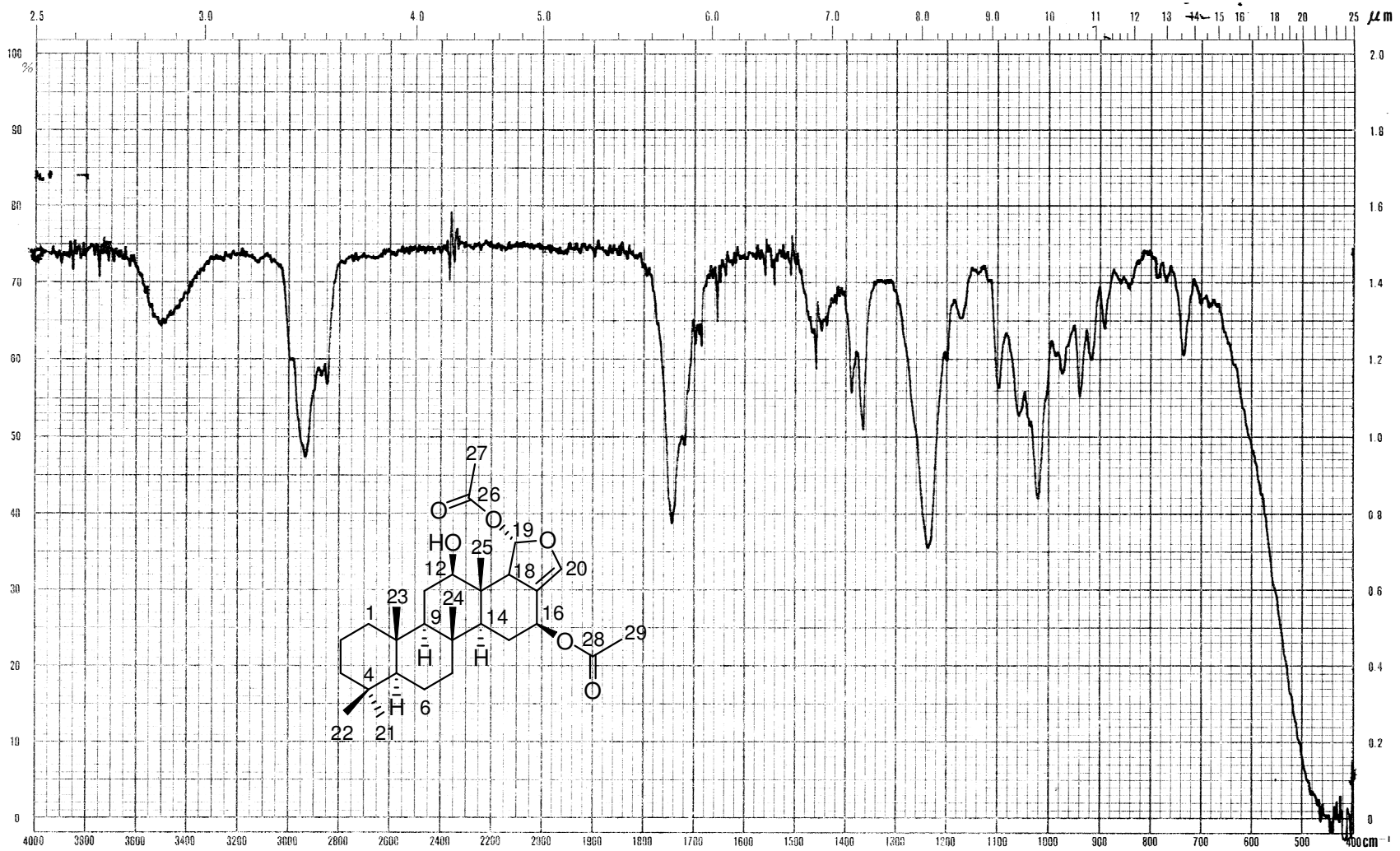


IR spectrum of **1** and **2**

^{13}C NMR spectrum of **1** and **2** (C_6D_6; 125 MHz)



UV spectrum of **3**



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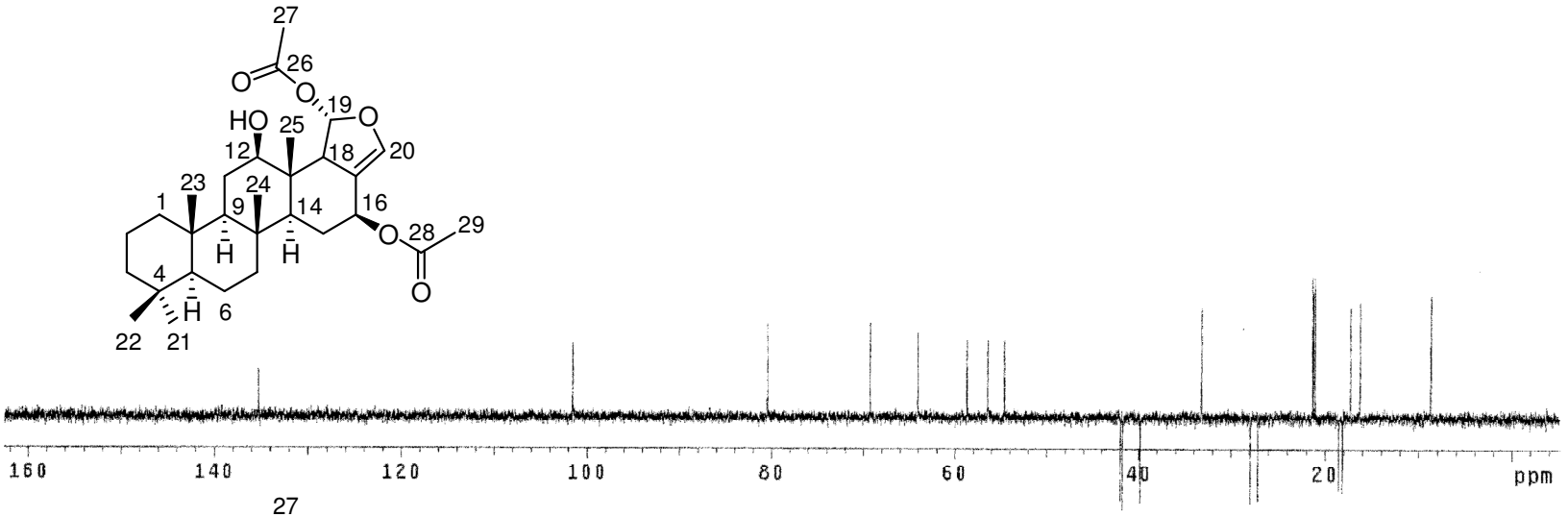
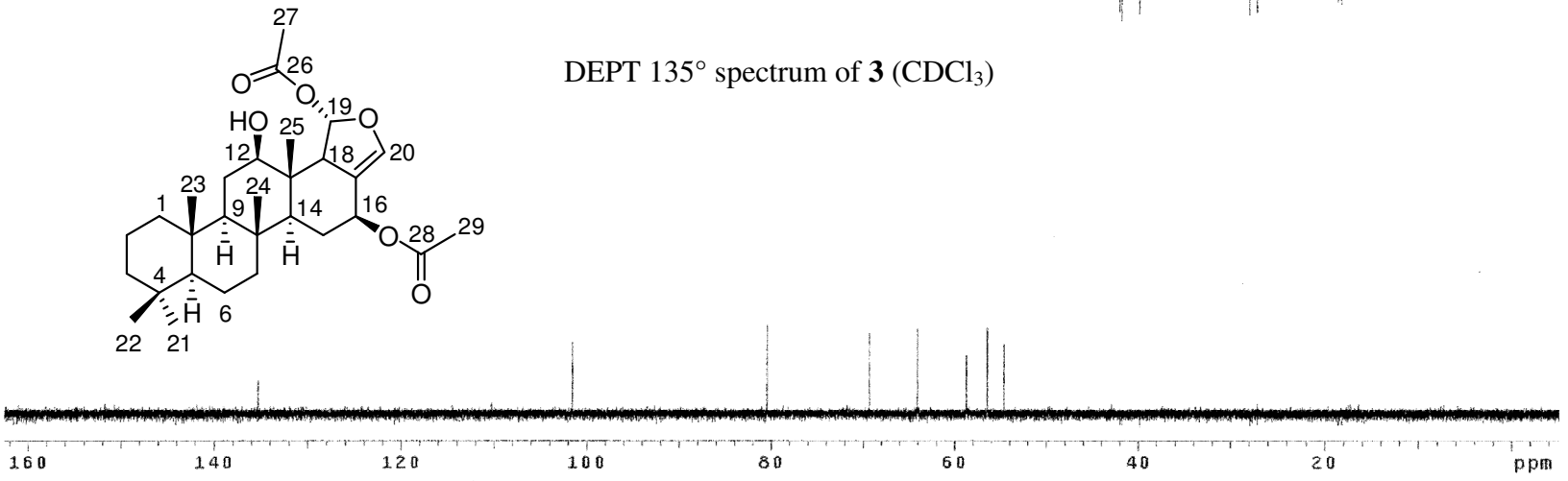


DEPT 135° spectrum of **3** ($CDCl_3$)



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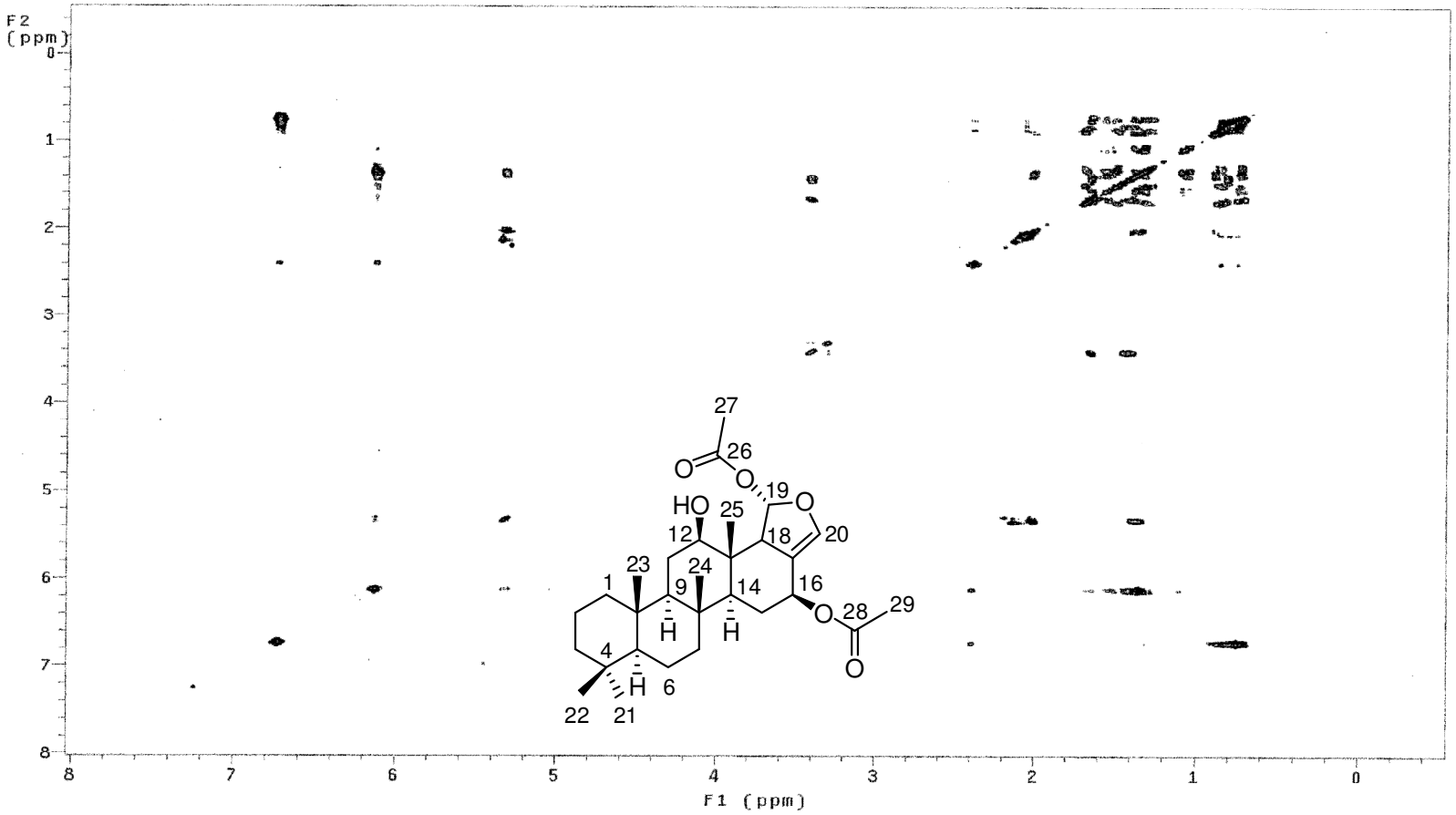
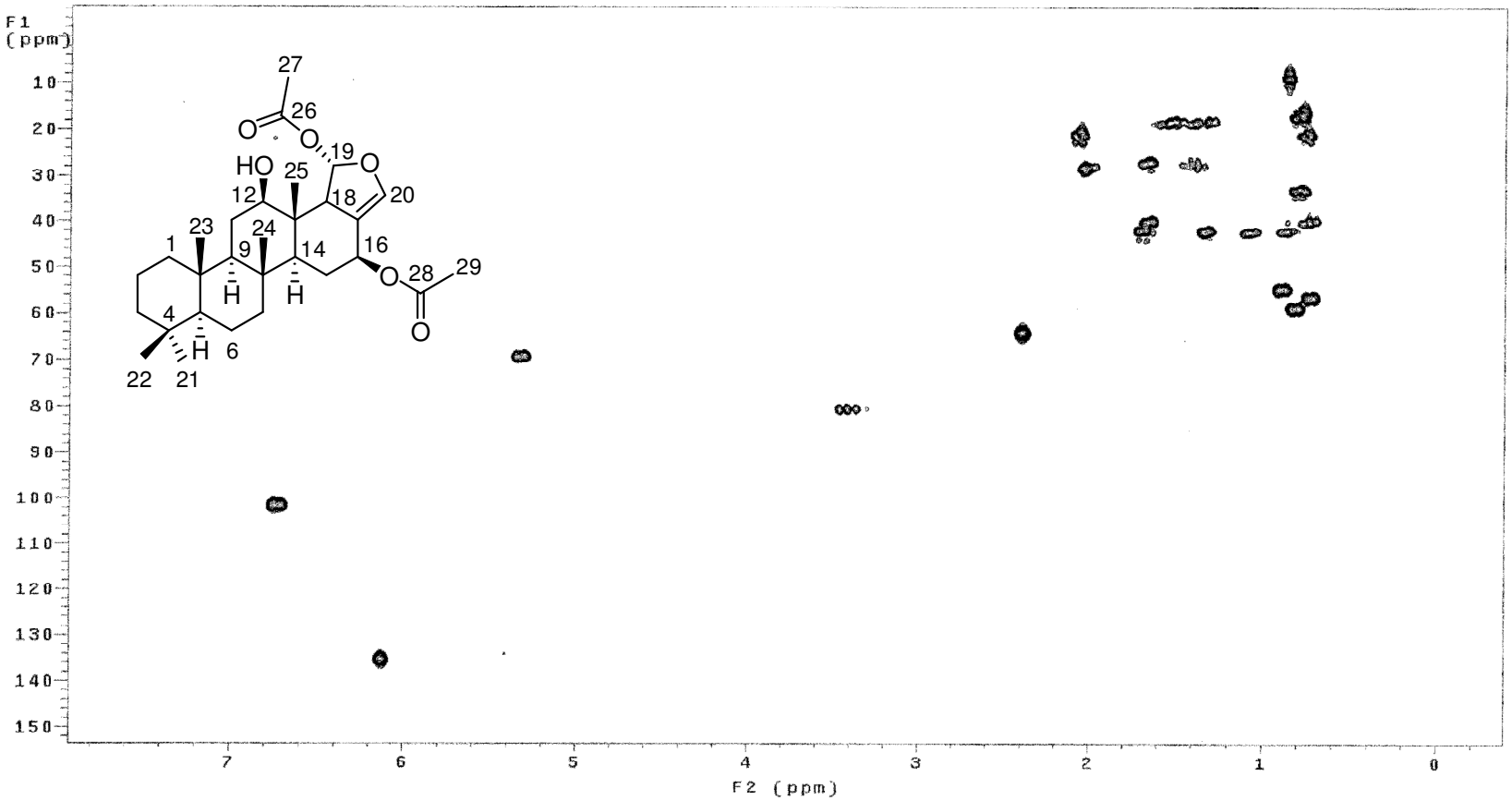
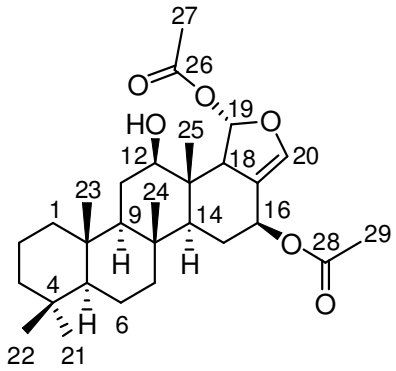


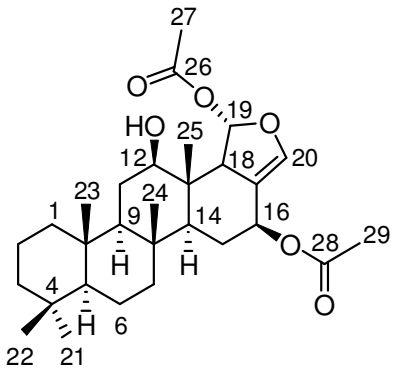
^{1}H-^{1}H COSY spectrum of **3** ($CDCl_3$)



HMQC spectrum of **3** ($CDCl_3$)



HMBC spectrum of **3** ($CDCl_3$)



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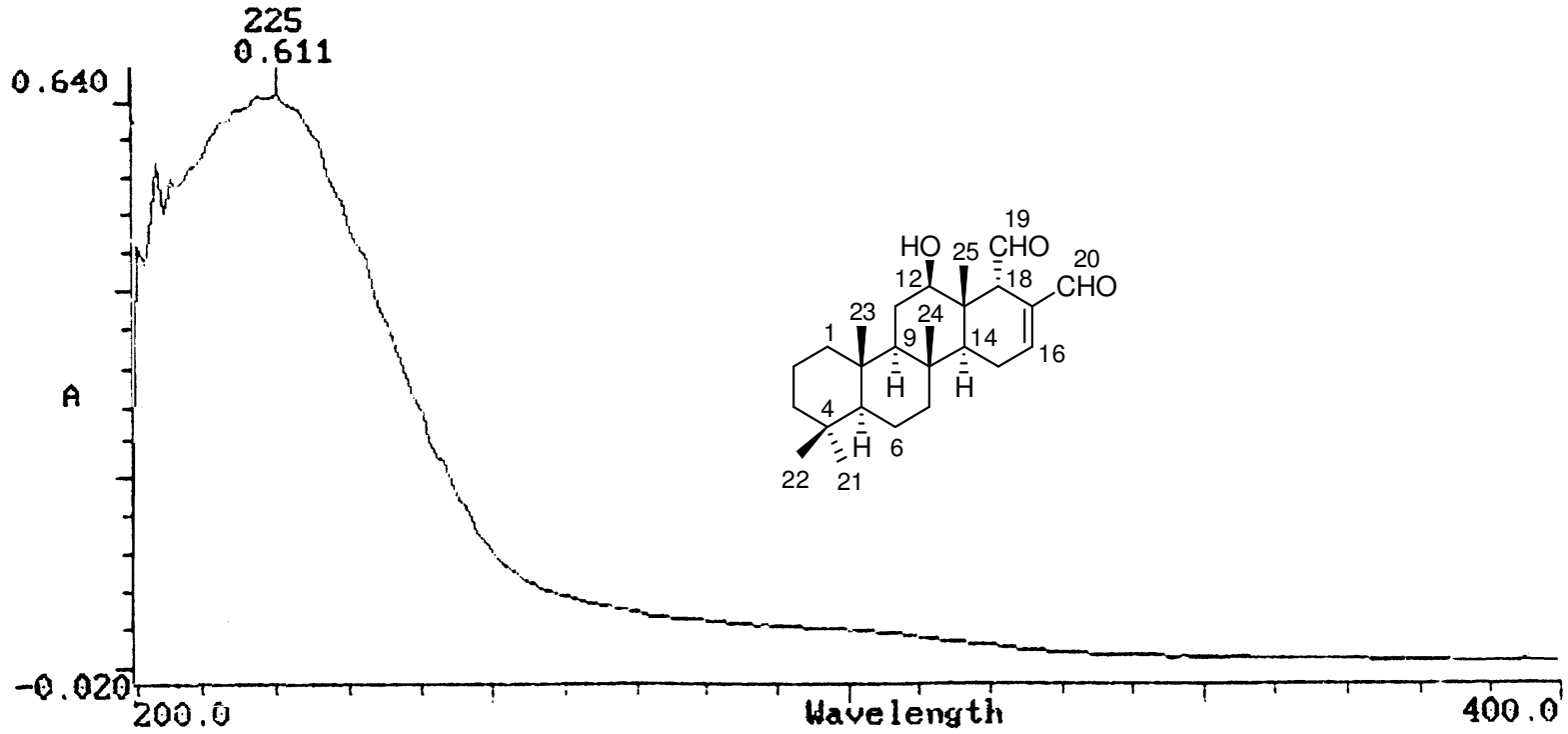
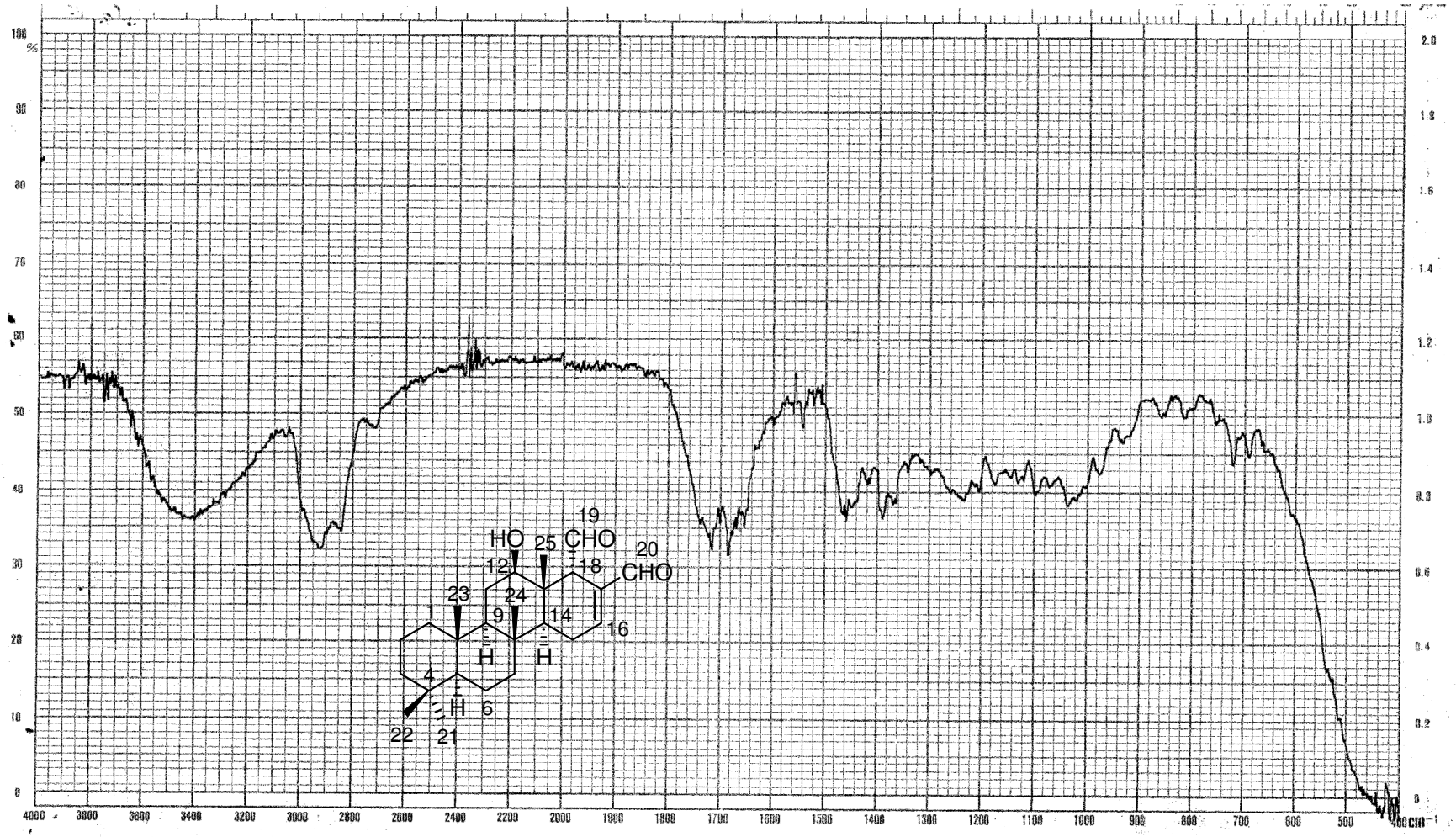


UV spectrum of **4**



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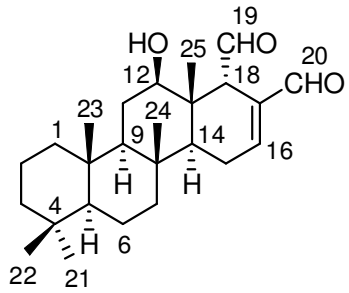
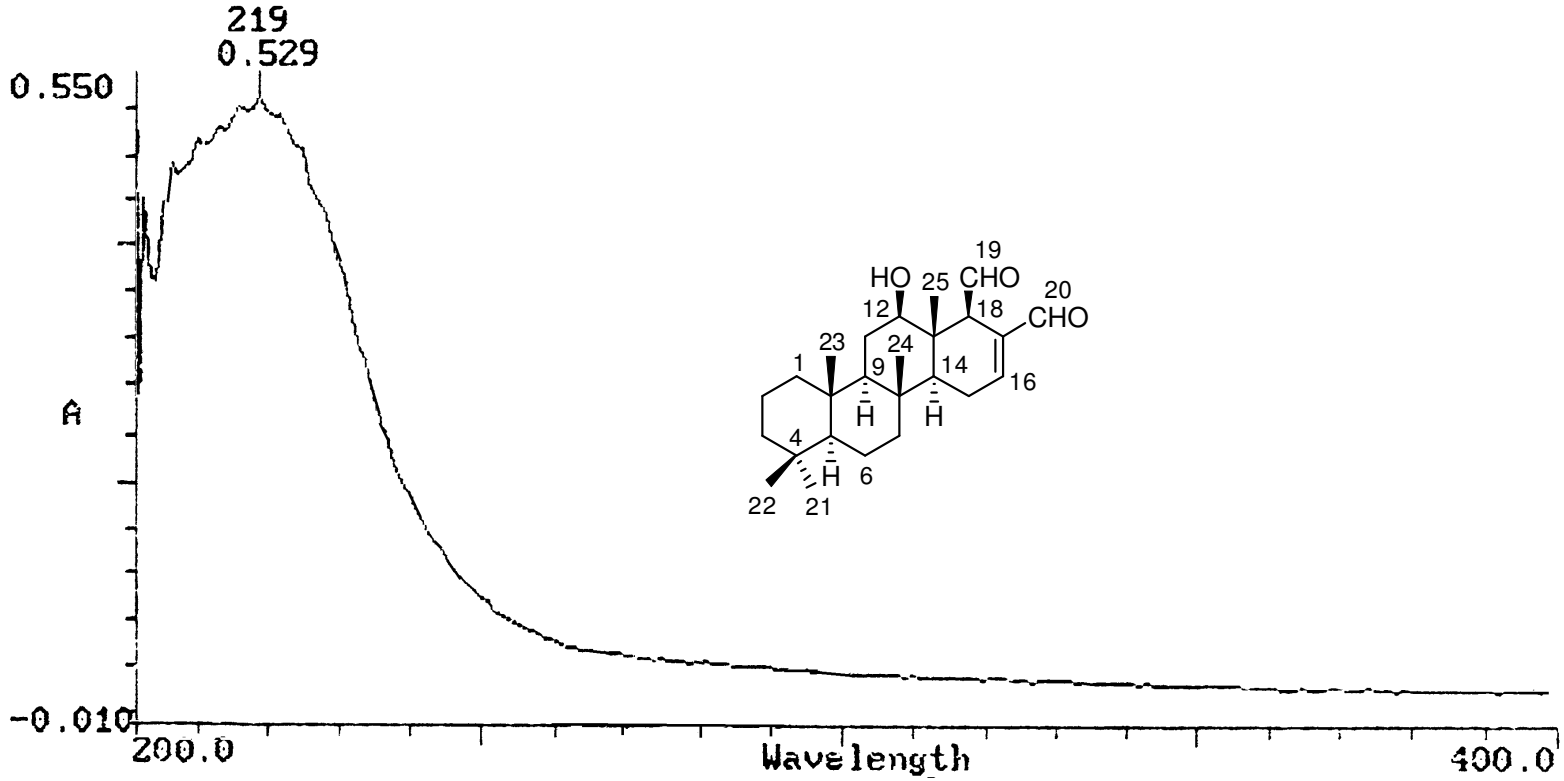
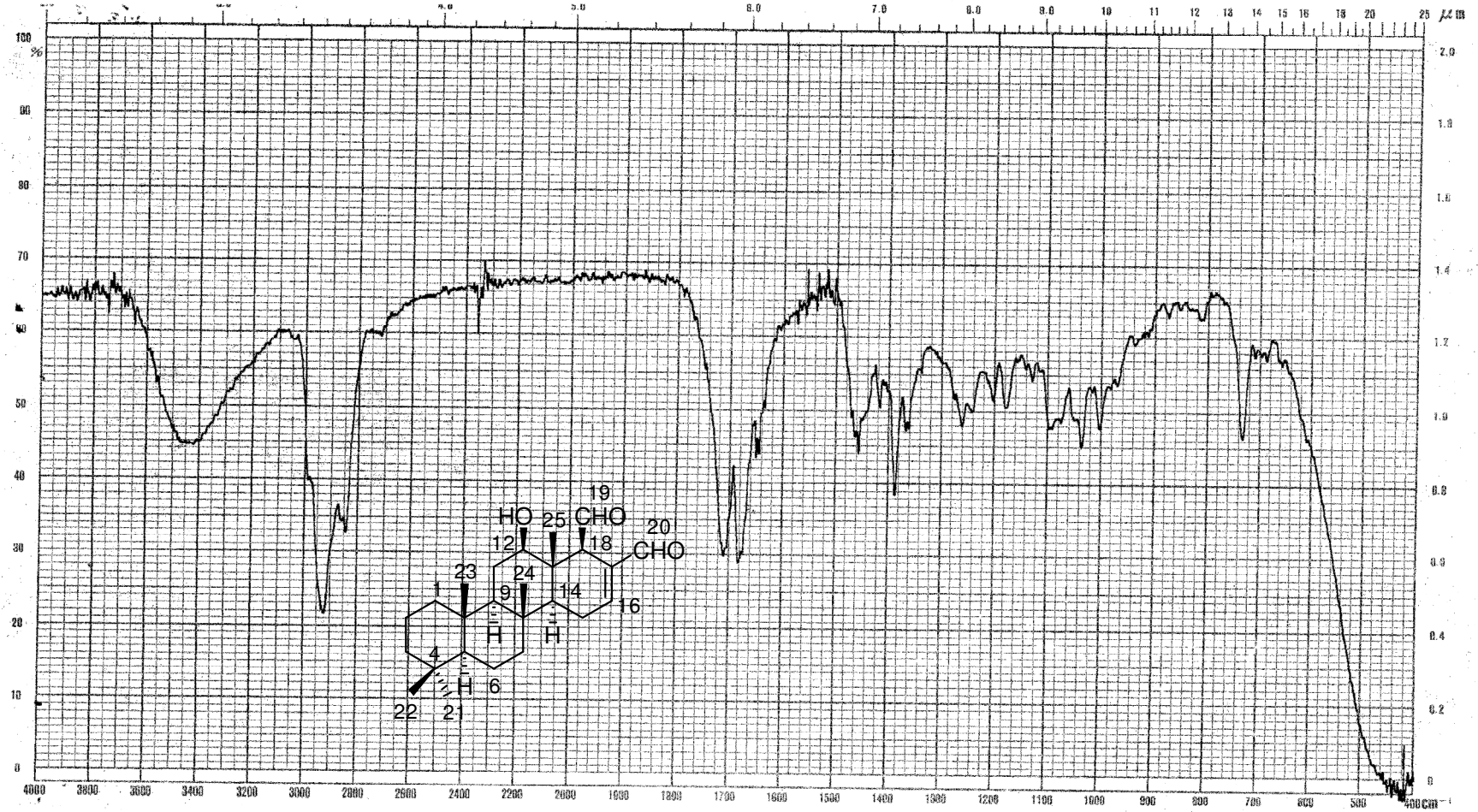


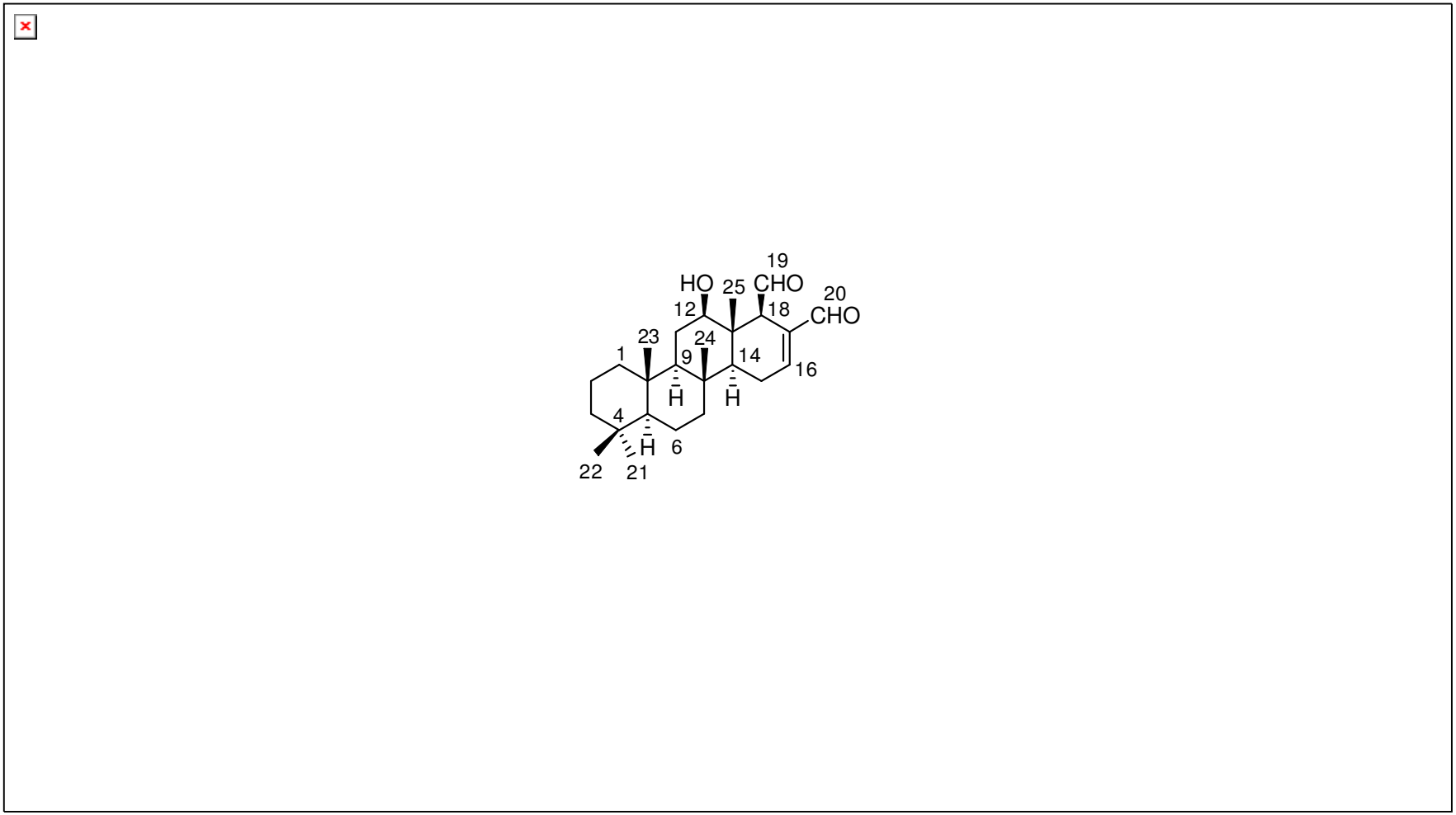
EI mass spectrum of **4**



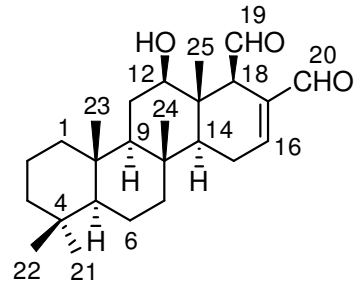
UV spectrum of **5**



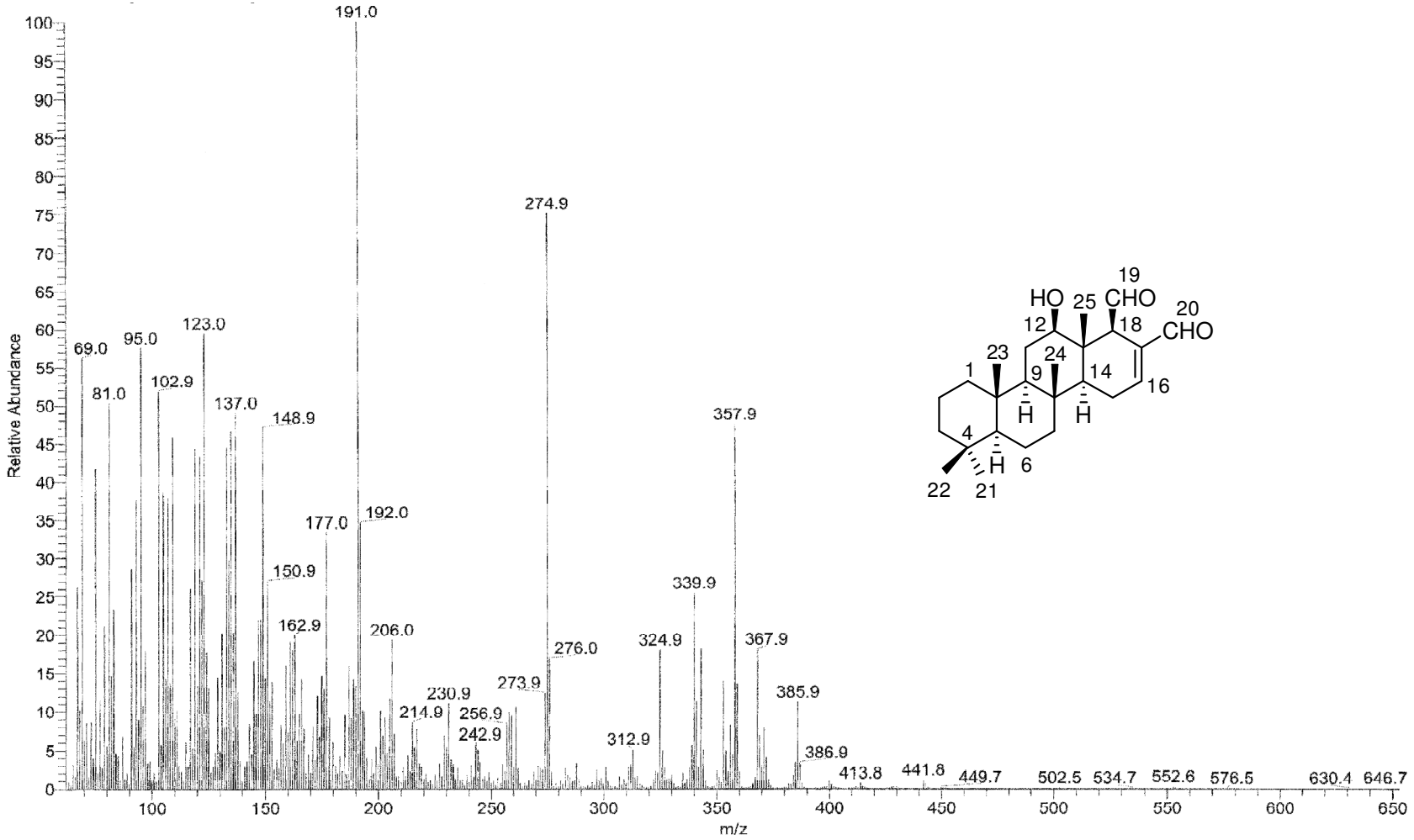
IR spectrum of **5**



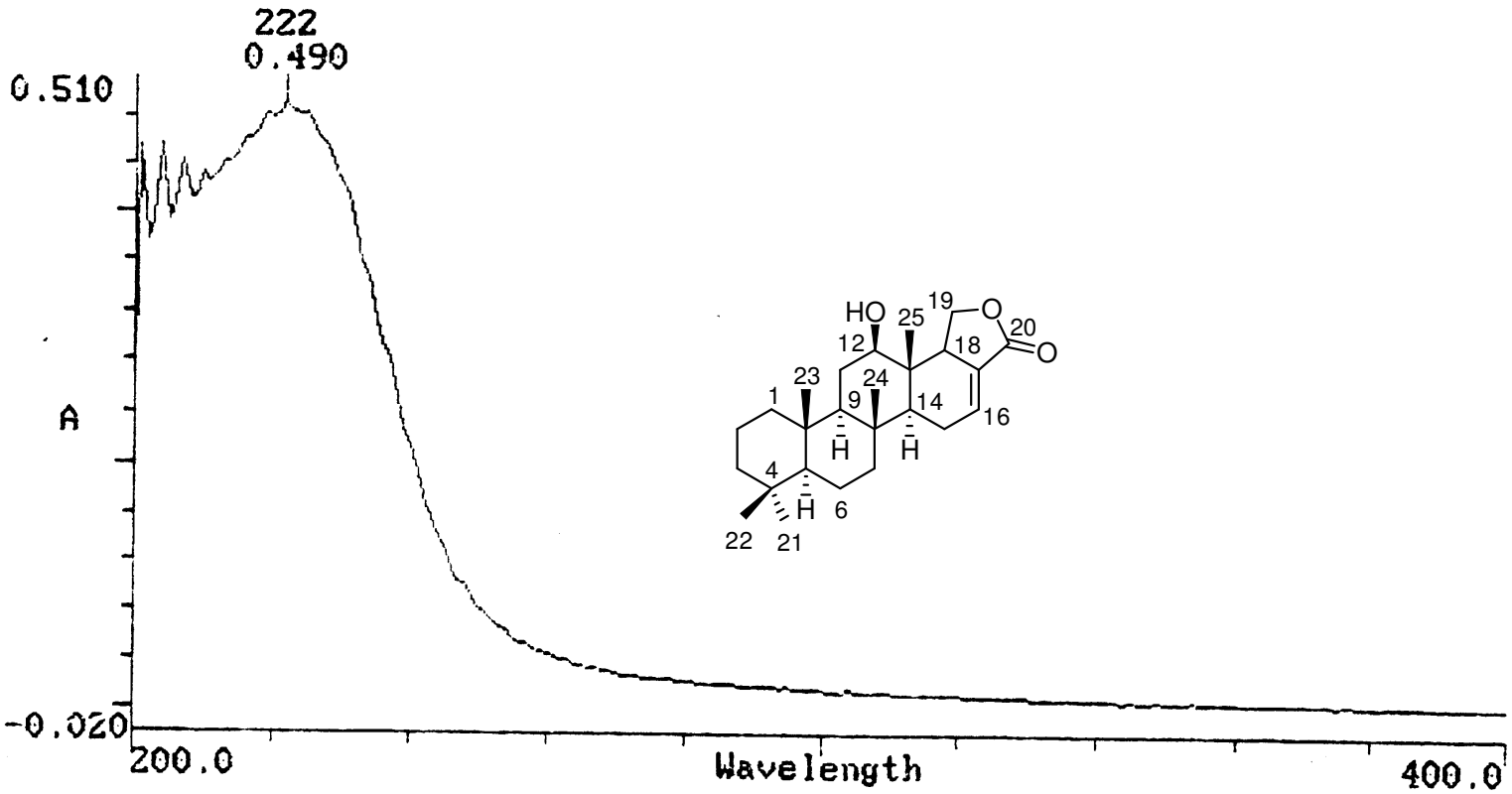
nOe difference spectrum of **5** after irradiation at δ_H 3.71 (H-12)



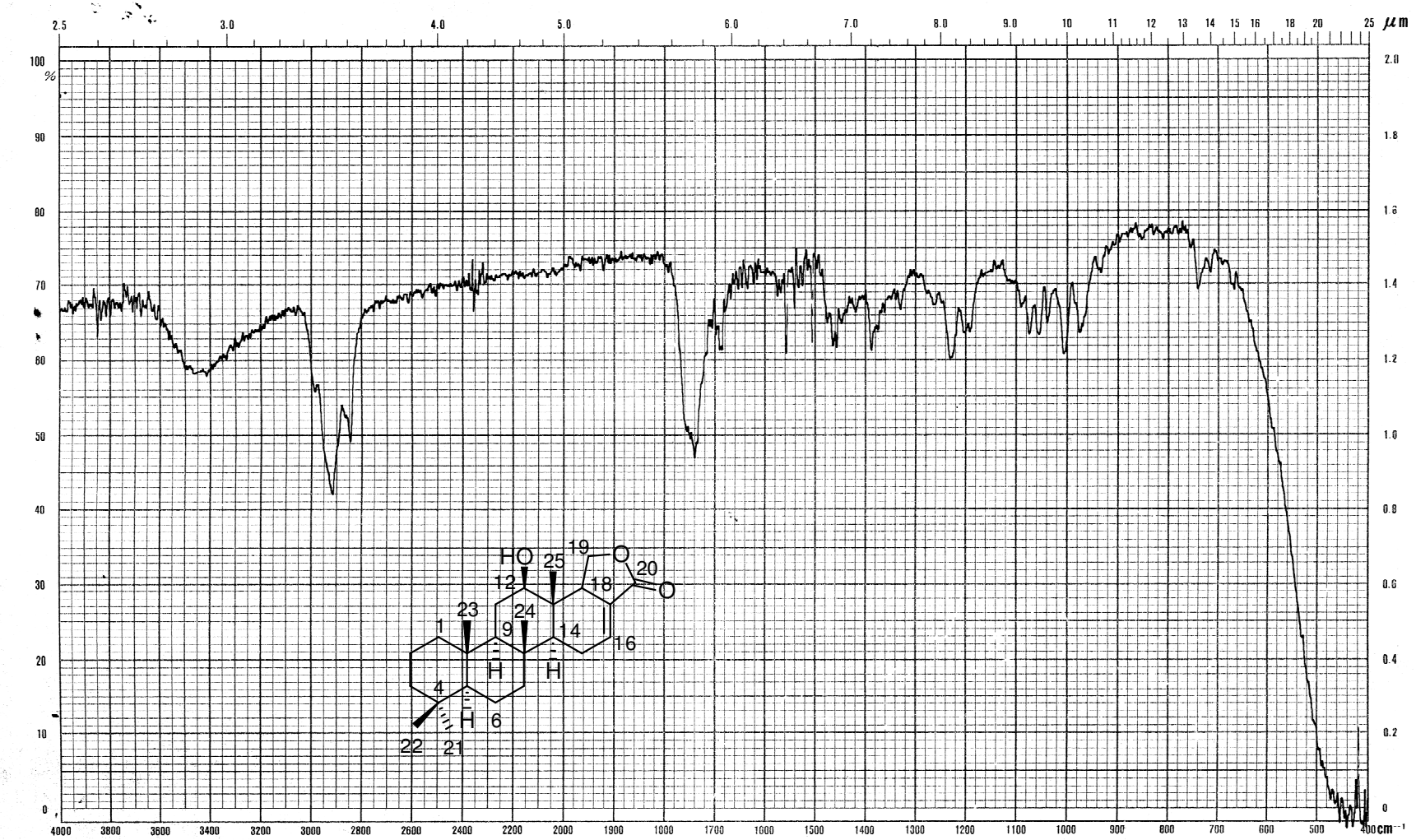
nOe difference spectrum of **5** after irradiation at δ_H 3.11 (H-18)



EI mass spectrum of **5**



UV spectrum of **6**



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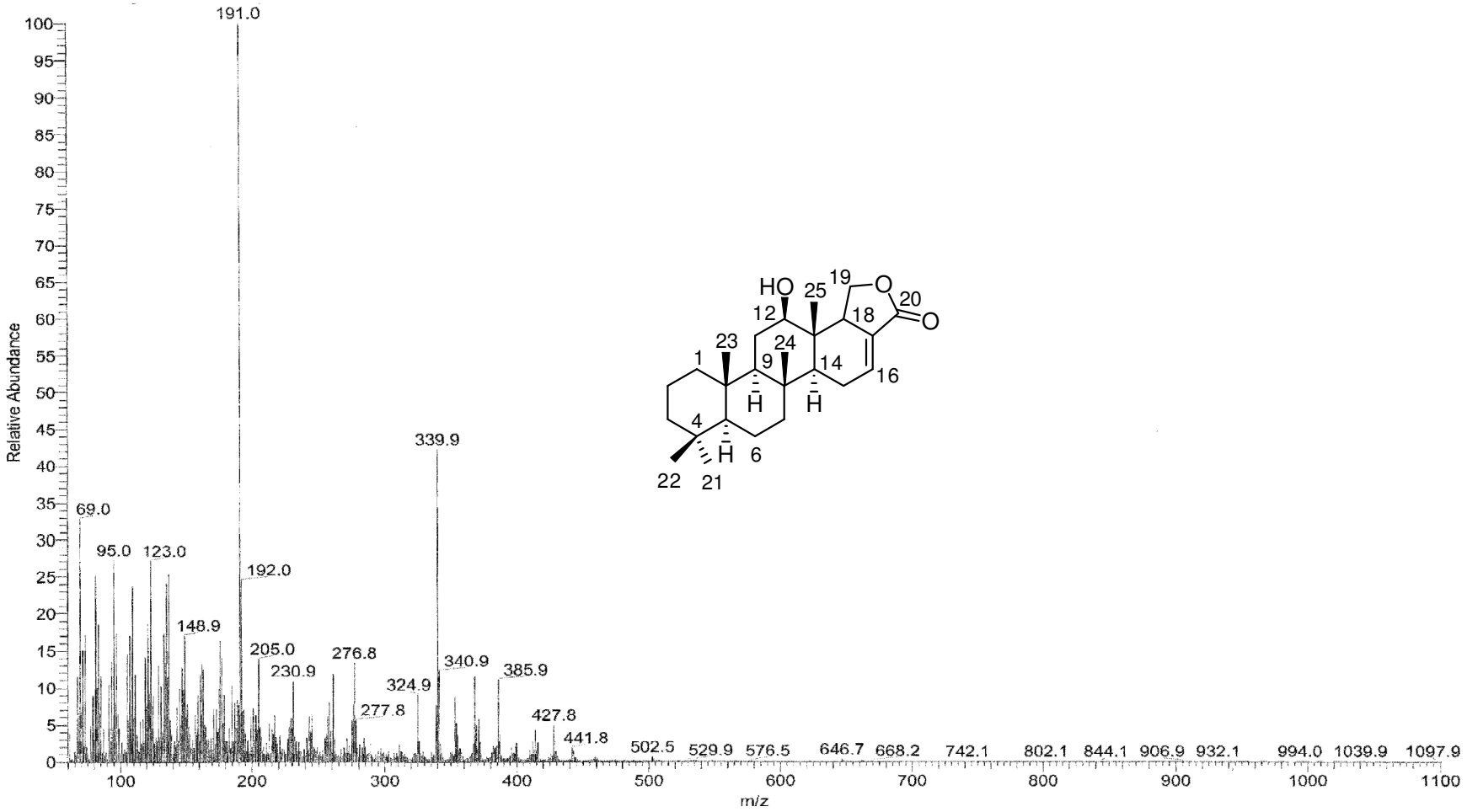

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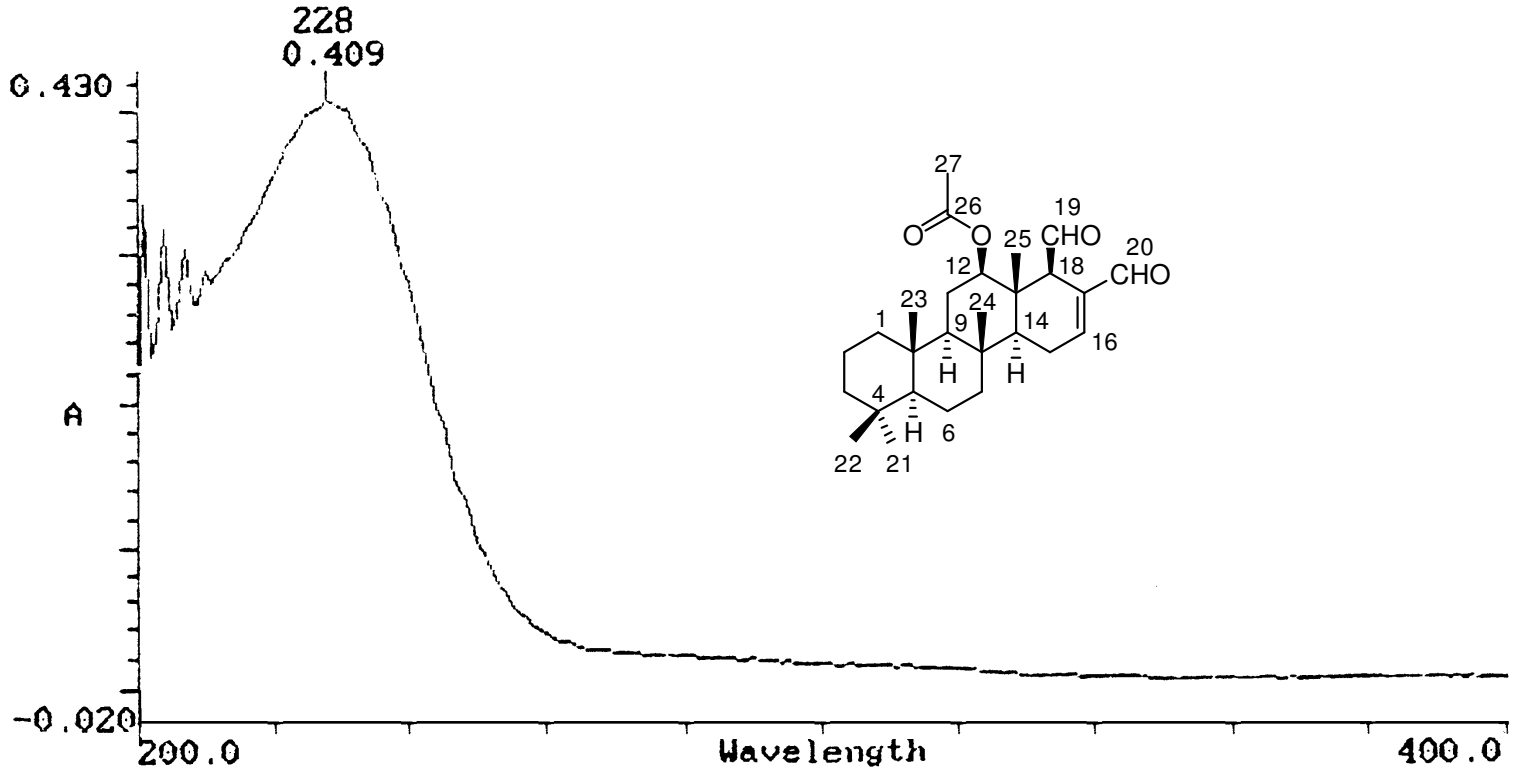
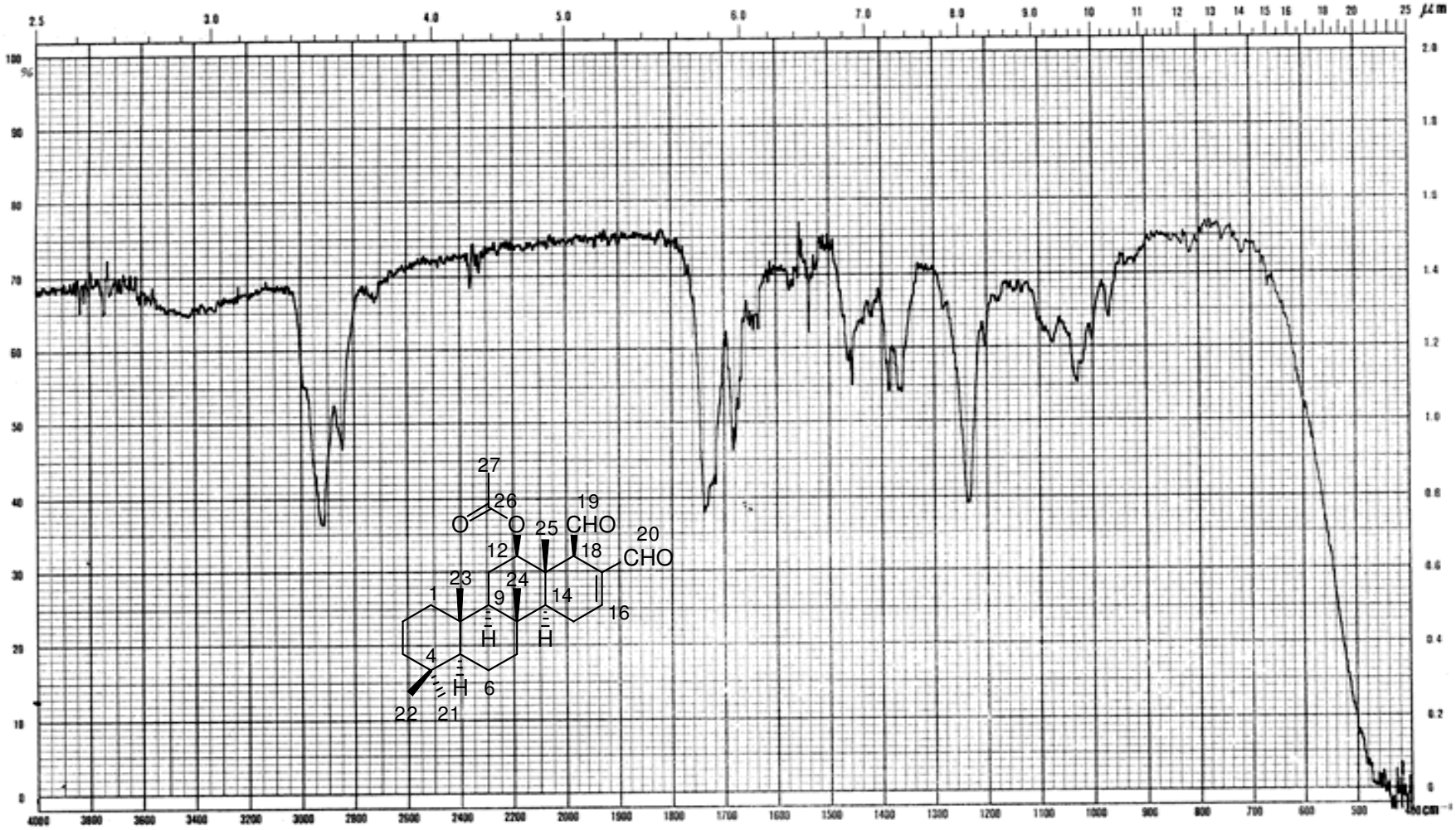
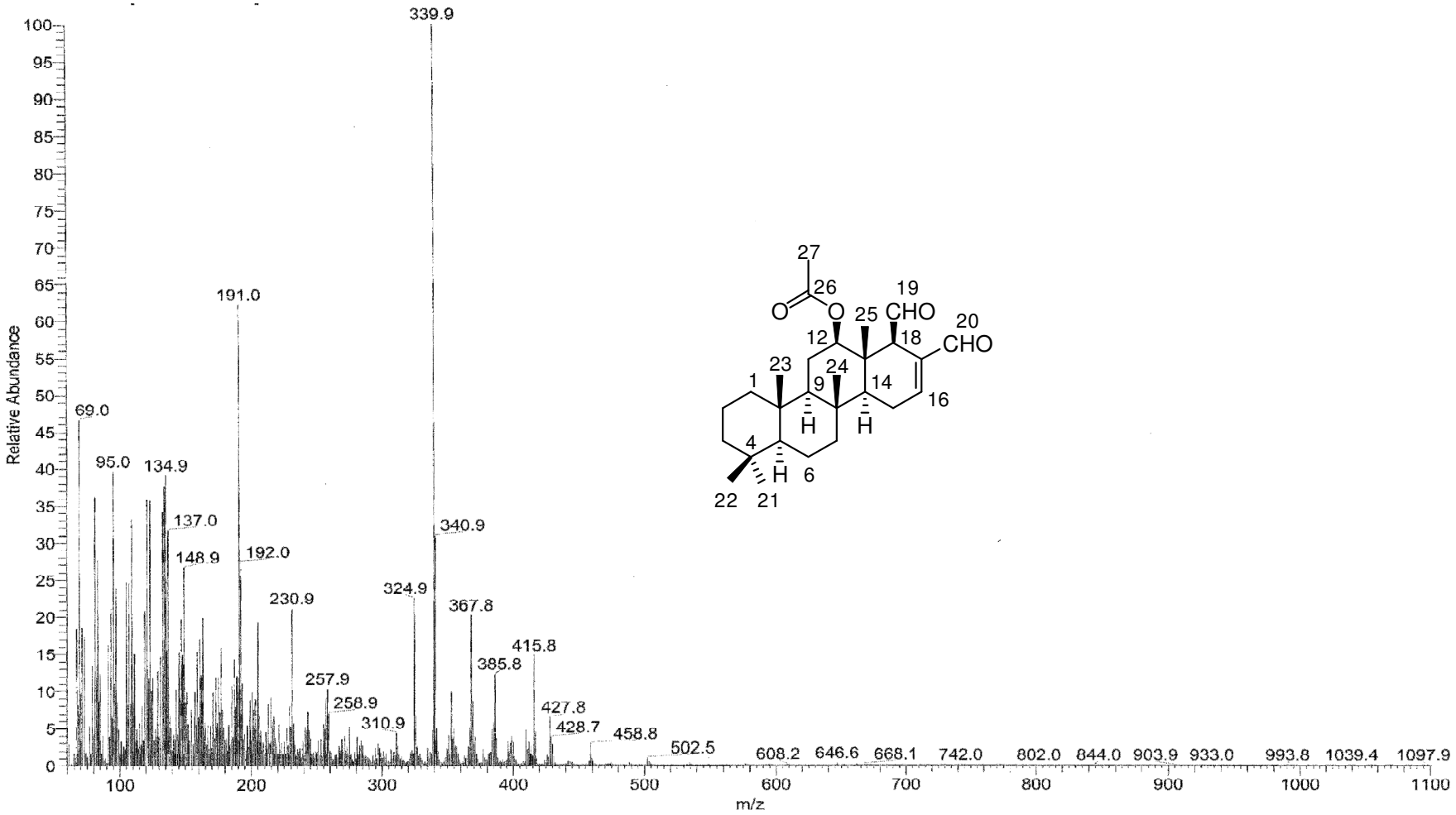


UV spectrum of **7**



IR spectrum of **7**



EI mass spectrum of **7**

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List of Publication and Proceedings

Phatchareephorn Chatakul, Chatchai Wattanapiromsakul and Anuchit Plubrukarn. 2010. Antimalarial and antitubercular activities of heteronemin isolated from the Thai marine sponge *Aplysinopsis* sp. *Proceeding of 3rd Silpakorn Research Conference*, Silpakorn University, Sanam Chan Palace, Nakhon Pathom, Thailand. P. 82-83.