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이학석사 학위논문

**Identification of Bioactive Secondary
Metabolites from Marine Organisms**

- 해양생물의 생리활성 이차대사산물 규명 -

August 2014

Laboratory of Marine Drug

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Identification of Bioactive Secondary Metabolites from Marine Organisms

- 해양생물의 생리활성 이차대사산물 규명 -

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이 논문을 이학석사 학위논문으로 제출함

2014년 6월

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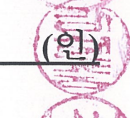
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위 원

남 상 집



Abstract

Marine natural products have been a potential sources of drug leads and new drugs. More than 70% of earth's surface is covered with the ocean and diverse marine organisms inhabit the ocean. Marine organisms living in the diverse environment have inspired researchers to identify unprecedented marine natural products which could be developed into therapeutics. Furthermore, technical improvements have accelerated exploration of the marine environment and development of the marine natural product chemistry.

In the course of our search for secondary metabolites from marine microorganisms, we obtained two novel compounds from CNQ675 strain (*Nocardiopsis* sp., La Jolla, California, USA) which showed potent inhibitory activity on nitric oxide synthesis. Their structures were elucidated on the basis of ^1H , ^{13}C , 2D NMR, MS, UV and IR analysis.

Four known sesquiterpenoids and diketopiperazines were isolated from Korean marine sponge derived fungi, *Aspergillus versicolor*, using bioassay-guided fractionation. Their structures were elucidated by the interpretation of ^1H , ^{13}C , 2D NMR, MS and UV spectroscopic data. These compounds exhibited significant anti-inflammatory activity by inhibiting the production of PGD_2 , LTC_4 .

Three known degraded sterol derivatives were isolated from a Korean marine sponge *Homaxinella* sp. The crude extract exhibited significant antibacterial activity selectively against Gram-positive strain (*Staphylococcus aureus* CCARM 0205).

Key words

Marine natural products, Secondary metabolites, Marine bacteria, Marine-derived fungi

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Chapter 1

Introduction

1.1 Marine Natural Products

Natural products have been a traditional source of drug discovery, such as penicillins from fungi and morphine from poppies. Pharmacologically active compounds from plants and microbes have been a prominent pipeline for new drugs.¹⁾

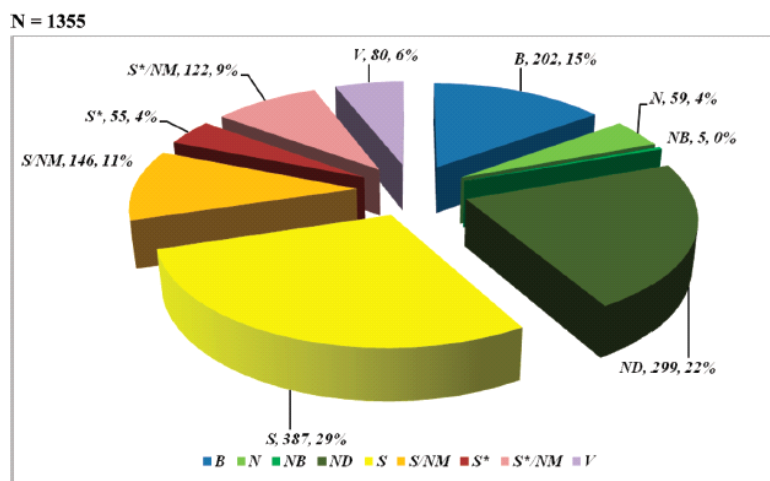


Figure 1.1 Source of approved new drugs, 1981-2010

"N", natural product. "ND", derived from a natural product and is usually a semi-synthetic modification. "NM", natural product mimic. "S", totally synthetic drug often found by random screening/modification of an existing agent. "S*", made by total synthesis, but the pharmacophore is/was from a natural product. "V", vaccine, "B", biologics, "NB", natural product botanical.²⁾

1) Molinski, T. F.; Dalisay, D. S.; Lievens, S. L.; Saludes, J. P., *Drug Discovery* **2009**, 8, 69-85

2) Newman, D. J.; Cragg, G. M. *J. Nat. Prod.* **2012**, 75, 311-335

Limited source of terrestrial organisms, however, causes discovery for natural products difficult and the role of natural product in pharmaceutical industry as a source of drug discovery was reduced.³⁾

More than 70% of earth's surface is covered with the ocean and diverse marine organisms inhabit the ocean. Marine organisms living in the diverse environment have inspired researchers to identify unprecedented marine natural products that could be developed into therapeutics. Marine organisms has generally soft bodies. Therefore, these organisms have developed the defense mechanisms that they synthesize toxic compounds or obtain them from marine microorganisms.⁴⁾ Furthermore, technical improvements for the easy accessibility of the ocean such as SCUBA, underwater walking robot and grab-sampler have accelerated study of marine natural product chemistry.

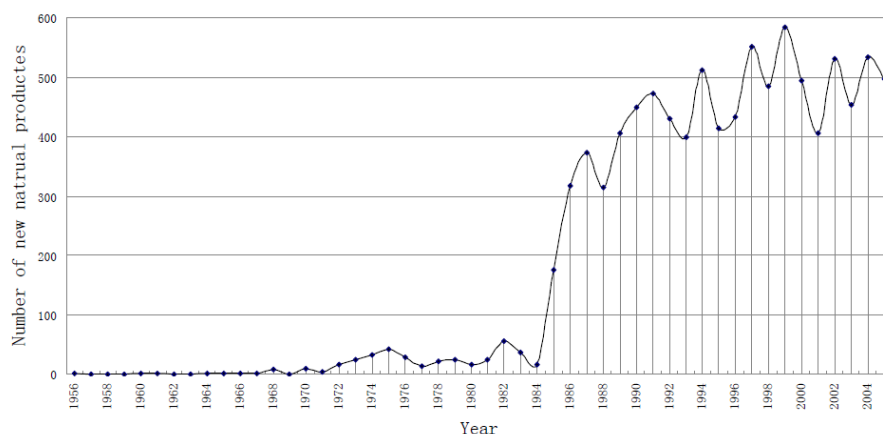


Figure 1.2 Temporal trend in the number of novel products obtained from marine organisms

More than 20,000 compounds have been discovered from marine organisms since the 1960s. Figure 1.2 showed a number of novel products obtained from

3) Newman, D. J. *J. Med. Chem.* **2008**, 51, 2589-2599

4) Haefner, B. *Drug Discovery Today* **2003**, 8, 536-544

marine organisms. In general, the exploitation of marine organisms to isolate novel products has developed rapidly since the mid-1980s.⁵⁾

Marine invertebrates, marine algae and marine microorganisms are three major classes for discovery of marine natural products between 1985 and 2008.

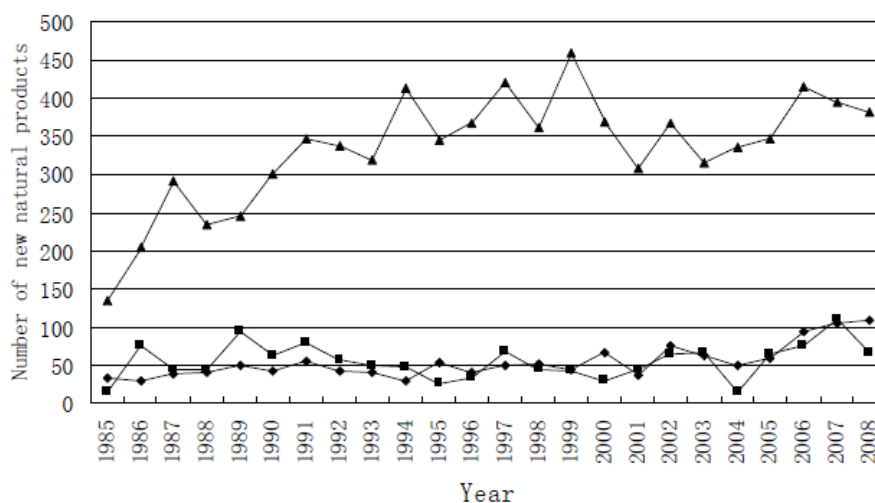


Figure 1.3 Temporal trends in the number of novel compounds isolated from different marine organisms.

Marine invertebrate (▲); Marine algae (■); Marine microorganisms (including phytoplankton)(◆).

Until 2008, the majority of novel compounds were isolated from marine invertebrates such as tunicate, sponge and coral. These marine invertebrates are most widely distributed in the world and inhabit the place with the richest biodiversity. However, it is difficult to recollect marine invertebrate on a large scale to obtain novel compound. Thus, supply problems emerge in the drug discovery process.

Marine algae are potential drug sources much earlier than marine

5) Hu, G. P.; Yuan, J.; Sun, Z. G.; Wu, J. H.; Lan, X. J.; Zhu, X.; Lin, Y. C.; Chen, S. P. *Mar Drugs*. 2011, 9, 514-525

microorganisms and marine invertebrate due to ease of collection in shallow waters. Most of the novel compounds was isolated from red algae, brown algae and green algae. These species of algae have been utilized on an large scale, which indicates that the novel compounds from marine algae are more appropriate as promising drugs than those from marine invertebrate. However, there has been a decline in the discovery of novel compounds from marine algae by reason of a lot of research.

Marine microorganisms are the potential sources for drug discovery. The number of novel compounds isolated from marine microorganisms has been increasing since 1997. Researchers have focused on the marine microorganisms for discovery bioactive secondary metabolites. Because microorganisms synthesize small molecules with potent biological properties and fascinating chemical structures and microorganisms are faster and easier to large scale cultivation than marine invertebrate and algae. Most of the compounds were isolated from marine microorganisms belonging to the fungi, actinomycetes and phytoplanktons. (Figure 1.4)

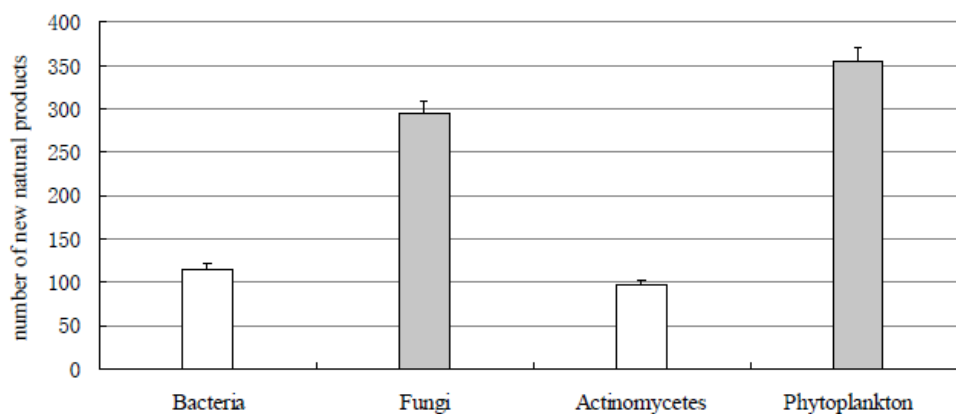


Figure 1.4 The number of novel compounds isolated from marine microorganisms between 1985 and 2008.

1.2 Marine Natural Products in Drug Discovery

Seven therapeutic agents are derived from the marine organisms and have been approved by Food and Drug Administration (FDA) or European Union. In addition, a further 13 compounds are undergoing phase I, II or III clinical trials.⁶⁾ (Table.1.1) ⁷⁾

Approved marine-derived drugs

Ziconotide (**1.1**) was originally isolated from the marine cone snail *Conus magus*. and has developed as a painkiller for chronic pain. A variety of marine natural products have been approved as anticancer drug or under the clinical trials. Trabectedin (Yondelis, **1.2**) was isolated from the tunicate *Ecteinascidia turbinata*, which has been the first marine anticancer agent approved in the European Union. Cytarabine (**1.3**) and vidarabine (**1.4**) are derivatives of nucleosides isolated from the Caribbean sponge *Terhya* sp. to treat human cancer and antiviral. Eribulin Mesylate (**1.5**) is a third marine anticancer agent from the sponge *Halicondria okadai* shows potent anticancer activity. Brentuximabvedotin (**1.7**) is the most recent addition of approved anticancer agents from the marine Mollusk *Dolabella auricularia*. (Figure 1.5)

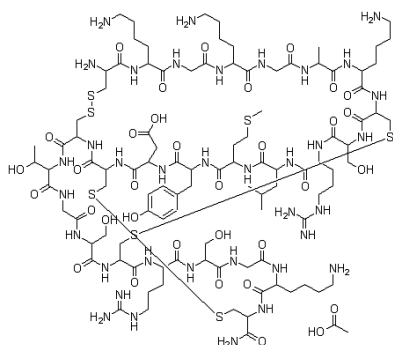
Marine pharmaceuticals : clinical pipeline

13 marine-derived compounds are in clinical development, as shown in Table 1.1.

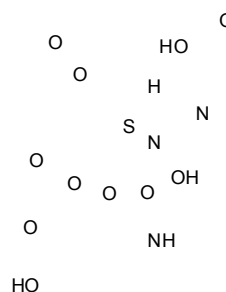
6) Mayer, A. M.; Glaser, K. B.; Cuevas, C.; Jacobs, R. S.; Kem, W.; Little, R. D.; McIntosh, J. M.; Newman, D. J.; Potts, B. C.; Shuster, D. E., *Trends Pharmacol. Sci.* **2010**, 31, 255-265

7) Gerwik, W. H.; Moore, B. S., *Chem. Biol.* **2012**, 19, 85-98

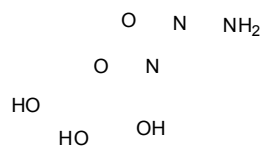
Plitidepsin (Aplidine, **1.8**) is a marine natural depsipeptide isolated from tunicate *Aplidium albicans*, currently in phase III trials and an potent inducer of apoptosis. (Figure 1.6)



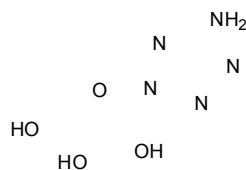
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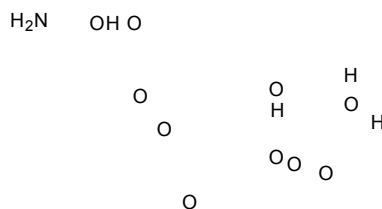
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1.3



1.4



1.5



1.6

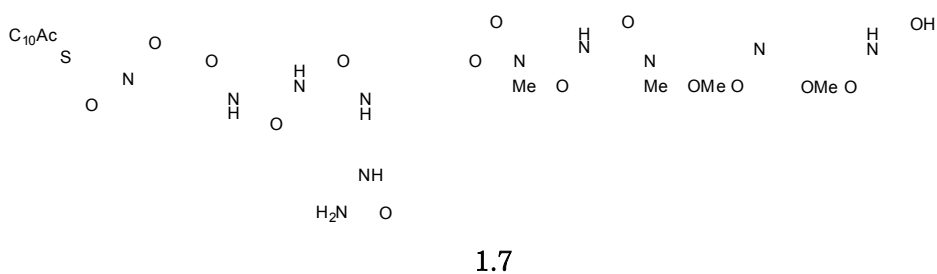


Figure 1.5 Approved marine drugs

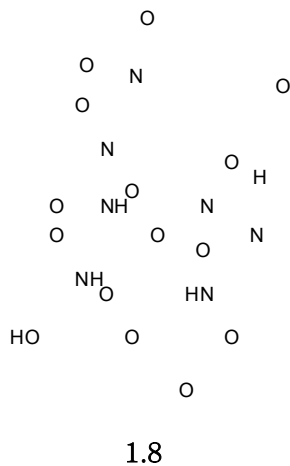


Figure 1.6 Marine-derived compounds in phase III trials

The marine natural products that are currently in phase II trials including DMXBBA (GTS-21, **1.9**), Plinabulin (NPI 2358, **1.10**), Zalypsis (PM00104, **1.11**), Glembatumumab vedotin (CDX-011, **1.12**) and Elisidepsin (**1.13**) as shown in figure 1.7.

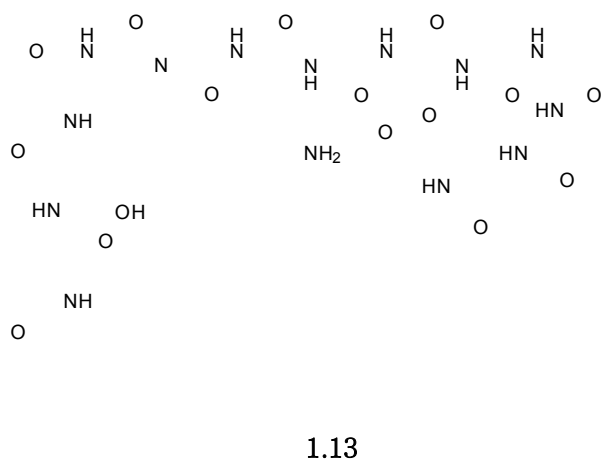
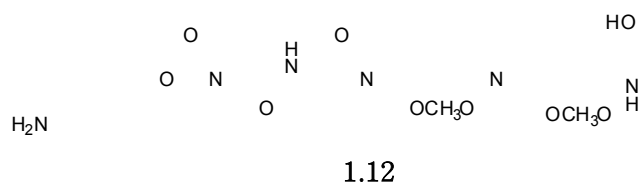
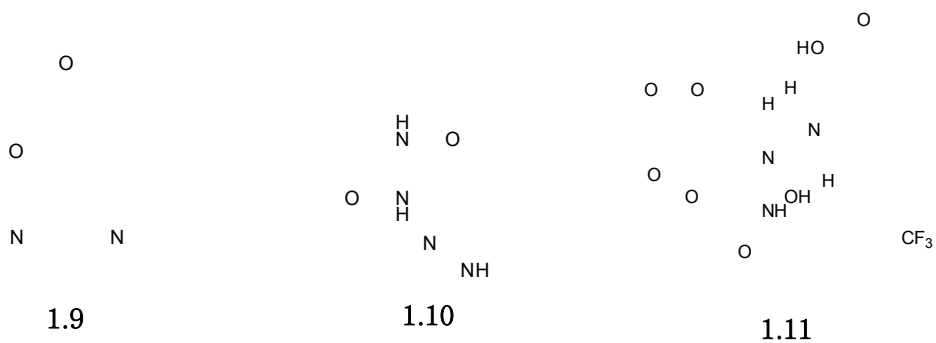


Figure 1.7 Marine-derived compounds in phase II trials

Marizomib (Salinosporamide A; NPI-0052, **1.14**), Trabectedin analog (PM01183), SGN-75, ASG-5ME, Hemiasterlin derivative (E7874, **1.15**), Bryostatin 1 (**1.16**) and Pseudopterosins are being investigated in phase I trials. (Figure 1.8)

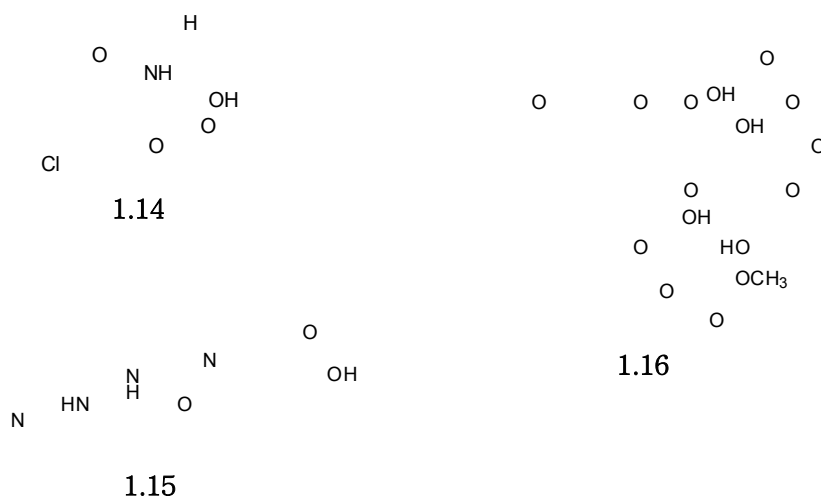


Figure 1.8 Marine-derived compounds in phase I trials

Table 1.1 Approved FDA drugs and clinical trial agents that were derived or inspired by marine natural products

Clinical Status	Compound Name	Natural Product or Derivative	Source Organism	Molecular Target	Disease Area
FDA approved	Cytarabine (Ara-C)	Derivative	Sponge	DNA polymerase	Cancer
	Vidarabine (Ara-A)	Derivative	Sponge	Viral DNA polymerase I	Antiviral
	Ziconotide	Natural Product	Cone Snail	N-type Ca channel	Pain
	Eribulin Mesylate (E7389)	Derivative	Sponge	Microtubules	Cancer
	Omega-3-acid ethyl esters	Derivative	Fish	Triglyceride-synthesizing enzyme	Hyper-triglyceridemia
	Trabectedin (ET-743) (EU registered only)	Natural Product	Tunicate	Minor groove of DNA	Cancer
	Brentuximab vedotin (SGN-35)	Derivative	Mollusk	CD and microtubules	Cancer
Phase III	Plitidepsin (Aplidine)	Natural Product	Tunicate	Rac 1 and JNK activation	Cancer
Phase II	DMXBA (GTS-21)	Derivative	Worm	$\alpha 7$ nicotinic acetylcholine receptor	Cognition Schizophrenia
	Plinabulin (NPI2358)	Derivative	Fungus	Microtubules and JNK stress protein	Cancer
	Elisidepsin	Derivative	Mollusk	Plasma membrane fluidity	Cancer
	Zalypsis (PM00104)	Derivative	Nudibranch	DNA binding	Cancer
	Glembatumumab vedotin (CDX-011)	Derivative	Mollusk	Glycoprotein NMB & microtubules	Cancer

Table 1.1 Continued

Phase I	Marizomib (Salinosporamide A; NPI-0052)	Natural Product	Bacterium	20S proteasome	Cancer
	Trabectedin analog (PM01183)	Derivative	Tunicate	Minor groove of DNA, Nucleotide Excision Repair	Cancer
	SGN-75	Derivative	Mollusk	CD70 and microtubules	Cancer
	ASG-5ME	Derivative	Mollusk	ASG-5 and microtubules	Cancer
	Hemiasterlin Derivative (E7974)	Derivative	Sponge	Microtubules	Cancer
	Bryostatin 1	Natural Product	Bryozoan	Protein kinase C	Cancer, Alzheimer's
	Pseudopterosins	Natural Product	Soft Coral	Eicosanoid metabolism	Wound healing

Six Marine Natural Products and Fourteen Marine Natural Products Inspired Compounds that Are FDA-Approved Agents or in Clinical Trial with Details of Their Collected Source, Molecular Target, and Disease Treated

1.3 The Future of Marine Natural Products

In the meantime, marine natural products discovery focused on isolation of novel compounds from macroorganisms, such as sponges and tunicates. However, Many compounds previously reported from macroorganisms are actually metabolic products of associated microorganisms. Furthermore, the producing marine macroorganisms are present in small natural abundance and difficult or impossible to recollect. Thus, supply problems emerge in the drug discovery process.

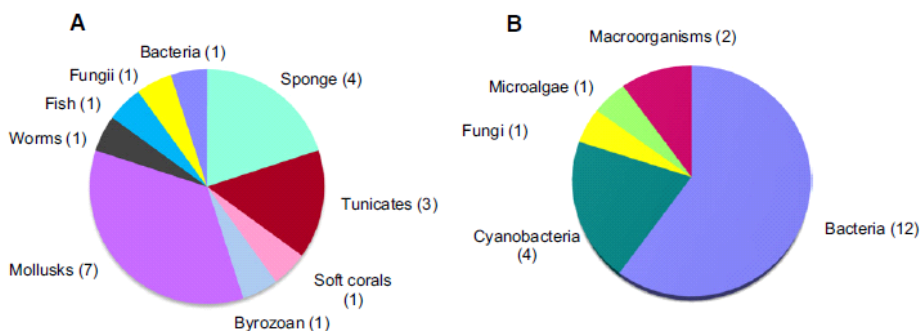


Figure 1.9 Pie charts illustrating the collected sources and predicted biosynthetic sources of marine derived or inspired drugs and clinical trial agents.

Figure 1.9 displays the marine-derived drugs and clinical trial agents divided by their subsequently shown or predicted source organisms, accounting for 80% of these agents are revealed from marine microorganisms. As a result, the research groups around the world have made an effort to culture marine bacteria or marine fungus from various sources, including shallow and deep water sediments and from within the tissues or surface of other macroorganisms.

In the course of our search for secondary metabolites from marine associated

microorganisms, we have isolated two new pyrone moiety compounds from marine bacteria, *Nocardiopsis* sp., four known sesquiterpenoids and diketopiperazines from marine associated fungi *Aspergillus versicolor* and three known degraded sterol derivatives from a marine sponge *Homaxinella* sp. Herein, we report the isolation, structure elucidation and biological evaluation of these marine natural products from diverse marine organisms.

Chapter 2

Marinopyrone D and E from a Marine-derived *Nocardiopsis* sp.

2.1 Introduction

Actinomycetes have been a promising source of structurally and functionally diverse bioactive compounds for several decades.⁸⁾ More than 15,000 bioactive molecules are produced by these microbes and many of them are used as drugs today. The study for discovery of new natural products has focused on terrestrial strains, however, there has been a decline in the discovery of new compounds from soil-derived actinomycetes in the past two decades. For this reason, the unexplored marine environments such as deep sea, sediment or polar ocean have been attractive for the study of marine actinomycetes or marine-derived actinomycetes in recent years.

Marine actinomycetes are virtually unlimited sources of novel compounds with many therapeutic applications due to their biological diversity and proven ability to produce novel bioactive compounds. Table 2.1 shows some examples of new secondary metabolites isolated from marine actinomycetes during last 5 years.⁹⁾

In the course of searching for new secondary metabolites from marine-derived actinomycetes, CNQ675 strain was obtained from marine sediment collected near-shore of La Jolla, California, which were seawater-dependent and shared 99.5 % 16S rRNA gene sequence identity with *Nocardiopsis* sp. We obtained two novel pyrone moiety compounds from CNQ675 that showed potent

8) Bérdy, J. J. *Antibiot.* **2005**, 58, 1 - 26

9) Subramani, R.; Aalbersberg, W. *Mircobiological Research* **2012**, 167, 571-580

inhibitory activity on nitric oxide production. The isolation, structural elucidation and biological activities of two compounds were described herein.

Table 2.1 A number of novel/new metabolites produced by marine actinomycetes during the period 2005-2010.

Compound	Source	Biological activity
Chinikomycins	Streptomyces sp.	Anticancer
Chloro-dihydroquinones	Novel actinomycete	Antibacterial; anticancer
Glaciapyrroles	Streptomyces sp.	Antibacterial
Frigocyclinone	Streptomyces griseus	Antibacterial
Lajollamycin	Streptomyces nodosus	Antibacterial
Mechercharmycins	Thermoactinomyces sp.	Anticancer
Salinosporamide A	Salinispora tropica	Anticancer; antimalarial
Sporolide A	Salinispora tropica	Unknown
Salinosporamides B & C	Salinispora tropica	Cytotoxicity
2-Allyloxyphenol	Streptomyces sp.	Antimicrobial
Saliniketal	Salinispora arenicola	Cancer chemoprevention
Marinomycins A-D	Marinispora	Antimicrobial; anticancer
Cyanosporaside A	Salinispora pacifica	Unknown
Arenimycin	Salinispora arenicola	Antibacterial; anticancer
Salinispyrone	Salinispora pacifica	Unknown
Salinipyrones A & B	Salinispora pacifica	Mild cytotoxicity
Pacificanones A & B	Salinispora pacifica	Antibacterial
Arenicolides A-C	Salinispora arenicola	Mild cytotoxicity
1-hydroxy-1-norresistomycin	Streptomyces chinaensis	Antibacterial; anticancer
Resistoflavin methyl ether	Streptomyces sp.	Antibacterial; antioxidative
Staurosporinone	Streptomyces sp.	Antitumor; phycotoxicity
Sesquiterpene	Streptomyces sp.	Unknown
1,8-Dihydroxy-2-ethyl-3-methylanthraquinone	Streptomyces sp.	Antitumor
Caboxamycin	Streptomyces sp.	Antibacterial; anticancer
Butenolides	Streptoverticillium luteovorticillatum	Antitumor
Daryamides	Streptomyces sp.	Antifungal; anticancer
Piericidins	Streptomyces sp.	Antitumor
Proximicins	Verrucosispora sp.	Antibacterial; anticancer
Streptokordin	Streptomyces sp.	Antitumor
ZHD-0501	Actinomadura sp.	Anticancer
Bisanthraquinone	Streptomyces sp.	Antibacterial
Tirandamycins	Streptomyces sp.	Antibacterial

2.2 Result and Discussion

2.2.1 Structural Elucidation

2.2.1.1 Marinopyrone D (1)

Marinopyrone D (1) was isolated as a colorless amorphous solid. Its molecular formula was assigned by HRFABMS m/z 237.1494 $[M+H]^+$ to be $C_{14}H_{20}O_3$, requiring five degrees of unsaturation.

The 1H NMR spectrum of **1** indicated one methoxy singlet (δ_H 3.87), two methyl singlet (δ_H 2.04, δ_H 2.04), one methyl doublet (δ_H 1.10, d, $J = 6.75$ Hz) and two olefinic protons (δ_H 6.38, d, $J = 15.4$ Hz, δ_H 6.50, dd, $J = 15.4$, 8.3 Hz). On the basis of the coupling constant of H-7 (δ_H 6.38) and H-8 (δ_H 6.75), *E*-geometry was assigned. The ^{13}C NMR and HSQC spectrum of **1** display five methyl groups, a methylene group, two sp^2 -hybridized methine groups, an aliphatic methine group, a methoxy group and five sp^2 -hybridized quaternary carbon atoms.

COSY correlations led to the establishment of alkyl chain moiety with five carbon unit [H-8/ H-9/ H-10/ H-11 and H-12]. Pyrone moiety was indicated by downfield-shifted carbon signals for C-2 (δ_C 165.9) and C-6 (δ_C 152.6) and interpretation of COSY and HMBC spectroscopic data. The connection between the alkyl chain and pyrone ring moiety was achieved by HMBC correlations from an olefinic proton H-7 (δ_H 6.38) to carbon C-5 (δ_C 109.6) and C-6 (δ_C 152.6). In addition, HMBC correlations observed from H-14 to C-4 meant that the methoxy group is attached to C-4.

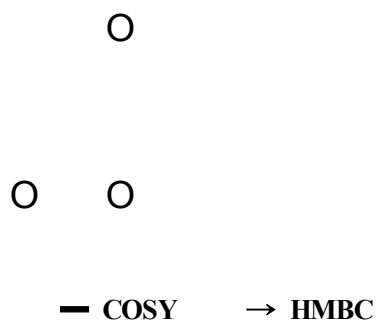
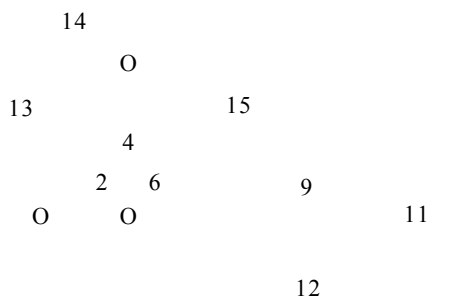


Figure 2.1 Structure of Marinopyrone D (**1**) with COSY (bold lines) and key HMBC (arrows) correlations

Table 2.2 Physical and spectral properties of marinopyrone D (**1**)

					Colorless amorphous solid
					Molecular formula : C ₁₄ H ₂₀ O ₃
					HRFABMS : <i>m/z</i> 236.1494
					IR (film) $\nu_{\max}$: 3418, 1681 cm ⁻¹
					UV (MeOH) $\lambda_{\max}$: 260, 350 nm

NMR Data^a

No.	δ_C , m ^b	δ_H , m, J(Hz)	COSY	HMBC
1				
2	165.9, C			
3	109.7, C			
4	169.3, C			
5	109.6, C			
6	152.6, C			
7	117.2, CH	6.38 (d, 15.4)	8	2, 5, 6, 8, 9
8	143.6, CH	6.50 (dd, 15.4, 8.3)	7, 9	6, 7, 9
9	38.9, CH	2.29 (m, 7.0)	8, 10, 12	7, 8
10	29.1, CH ₂	1.46 (m, 7.4)	9, 11	7
11	10.7, CH ₃	0.93 (t, 7.48)	10	9, 10
12	18.7, CH ₃	1.10 (d, 6.75)	9	7, 9, 10
13	8.9, CH ₃	2.04 (s)		2, 3, 4, 5, 6
14	59.7, CH ₃	3.87 (s)		4,
15	8.2, CH ₃	2.04 (s)		2, 3, 4, 5, 6

^a 1D and 2D NMR experiments were performed at 700MHz. ^b The Numbers of attached protons were determined by 1D and 2D spectroscopic data.

2.2.1.2 Marinopyrone E (2)

Marinopyrone E (2) was isolated as a colorless amorphous solid. Its molecular formula was assigned by LRMS m/z 223.1 $[M+H]^+$ to be $C_{13}H_{18}O_3$, requiring five degrees of unsaturation. It was also showed a UV absorptions at 260nm and 350nm. 1H NMR data of 2 was almost same as compound 1. The only difference occur in the side chain. The multiplet methylene at δ_H 1.46 disappeared and a triplet methyl [δ_H 0.93] of compound 1 was replaced a doublet methyl [δ_H 1.12] in the 1H NMR spectrum of compound 2. Also, it has a 14 mass units of difference from compound 1, which confirmed the compound 2 missed a CH_2 group from compound 1.



Mass spectrum of compound 13. The x-axis represents the mass-to-charge ratio (m/z) from 0 to 14, and the y-axis represents relative intensity from 0 to 100. The base peak is at m/z 4. Other significant peaks are labeled at m/z 2, 6, 9, 10, 11, 12, and 13.

Colorless amorphous solid

Molecular formula : C₁₃H₁₈O₃

LRMS : m/z 222.1

UV (MeOH) λ_{max} : 260, 350 nm

No.	δ_{H} , m, J(Hz)
1	
2	C
3	C
4	C
5	C
6	C
7	CH 6.38 (d, 15.4)
8	CH 6.60 (dd, 15.4, 8.3)
9	CH 2.55 (m)
10	CH ₃ 1.12 (d, 6.8)
11	CH ₃ 1.12 (d, 6.8)
12	CH ₃ 2.04 (s)
13	-OCH ₃ 3.88 (s)
14	CH ₃ 2.04 (s)

– 21 –

2.2.2 *i*NOS Inhibitory Activity of Marinopyrone D

Marinopyrone D (**1**) showed potent inhibitory activity on nitric oxide synthesis in LPS-activated mouse macrophage RAW264.7 cells with IC_{50} value of 13 μ M as shown in Figure 2.2.

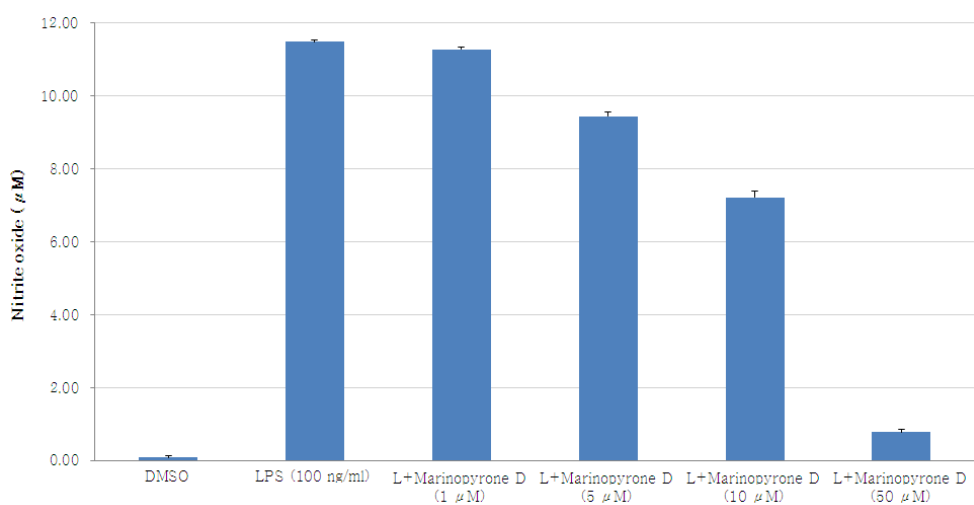


Figure 2.2 *i*NOS assay result of marinopyrone D (**1**)

2.3 Experimental

2.3.1 Instruments and Data Collection

^1H , ^{13}C and 2D NMR spectra were obtained using Bruker Advance 700MHz spectrometer with methanol- d_4 . The optical rotation was measured using a 5.0 cm cell on a Rudolph Research Autopol III. CD spectra were taken in an Applied Photophysics Chirascan plus CD spectrometer with a 0.5 mm pathlength rectangular cuvette. The UV spectrum was obtained with a Scinco UVS-2100 with a path length of 1 cm. IR spectra were recorded on a Thermo Electron Corp. EI-MS and FAB-MS were measured using a JEOL, JMS-AX505WA mass spectrometer. Low resolution LC-MS data were recorded on an Agilent Technologies 6120 quadrupole LC/MS system with a reversed-phase C18 column (Phenomenex luna 5 μ (2), 4.6 mm X 50 mm, 5 μm) at a flow rate of 1.0 mL/min. Purification of compounds by HPLC WATERS 1525 binary HPLC pump, WATERS 2489 UV/visible detector was carried out using reversed-phase semipreparative C₁₈ column (Phenomenex luna 5 μ (2), 250 X 10 mm, 5 μm). Solvent used in partitioning were first grade products of Dae-Jung & Metals Co., Korea. HPLC grade solvent from Burdick & Jackson were used in TLC and HPLC. NMR solvents were purchased from Cambridge Isotope Laboratories (CIL), Inc.

2.3.2 Bacterial Strain

Nocardiopsis sp. CNQ675 was obtained from marine sediment collected near-shore of La Jolla, California, USA. The 16S rRNA gene sequence using universal primers 27F and 1492R showed 99.5 % (1407/1410) similarity to *Nocardiopsis* sp. CNQ-327_SD01 (EU214917.1).



Figure 2.3 Picture of the bacterial strain, *Nocardiopsis* sp.

2.3.3 Fermentation, Extraction and Isolation

The bacterial strain CNQ675 was cultured at 25 °C with shaking at 130 rpm in 40 L Pyrex flask each containing 1 L of the medium SYP (10 g of starch soluble, 4 g of yeast extract, 2 g of peptone, dissolved in 1L filtered-seawater). After 8 days, the broth was extracted two times with ethyl acetate and evaporated to yield 2.1 g. of crude extract. The crude extract was subjected to medium pressure liquid chromatograph using step-gradient eluent mixture of dichloromethane and MeOH to afford 9 fractions (Fr 1 - Fr 9). Fraction 1 (20.9 mg) was further purified by C₁₈ reversed-phase HPLC (Phenomenex luna 5 μ (2), 250 X 10 mm, 5 μ m, 2.5 ml/min, UV = 210 nm), eluting with 70 % CH₃CN in H₂O to afford marinopyrone D (**1**, 14.0 mg) and marinopyrone E (**2**, 1.0 mg)

2.3.4 iNOS Assay

The iNOS inhibitory assay was performed for marinopyrone D (**1**) by measuring NO production in RAW 264.7 LPS-induced mouse macrophage cells. The cells were maintained in Dulbecco's modified Eagle medium (DMEM) supplemented with 1 % penicillin-streptomycin and 10 % fetal bovine serum (FBS) at 37 °C, in 5 % CO₂ humidified air. To evaluate the inhibitory activity of test materials on LPS-induced NO production, the cells in 10 % FBS DMEM were plated in 96-well plates (3×10^5 cells/mL) and then incubated for 24 hours. After incubation, the cells were further incubated in the medium with 100ng/mL of LPS in the presence or absence of test samples. After an additional 20 hours incubation, the media were collected and analyzed for nitrite accumulation as an indicator of NO production by the Griess reaction. Briefly, 100 μ L of Griess reagent [0.1 % N-(1-naphthyl)ethylenediamine dihydrochloride in H₂O and 1% sulfanilamide in 5% H₃PO₄] was added to 100 μ L of each supernatant from LPS or sample-treated cells in 96-well plates. The absorbance was measured at 540 nm using a microplate reader, and nitrite concentration was determined by comparison with a sodium nitrite standard curve. The percentage inhibition was expressed as $[1 - (\text{NO level of test samples} / \text{NO level of vehicle - treated control})] \times 100$.¹⁰⁾

10) Wang, W.; Lee, Y.; Lee, T. G.; Mun, B.; Giri, A. G.; Lee, J.; Kim, H.; Hahn, D.; Yang, I.; Chin, J.; Choi, H.; Nam, S. - J.; Kang, H. *Org. Lett.* **2012**, *14*, 4486 - 4489

Chapter 3

Bioactive Metabolites from Marine Sponge-derived Fungus

Aspergillus versicolor

3.1 Introduction

Marine natural products have been a prominent sources of drug leads and new drugs. Marine natural product approved drugs are derived from marine invertebrates such as soft coral, tunicates and sponges. These marine invertebrates are well known for a containing diverse microorganism communities. These communities are composed numerous microorganisms within their tissues including cyanobacteria, algae, bacteria, achaea and fungi which may exhibit the chemical defense of their hosts against pathogens by production of bioactive secondary metabolites.¹¹⁾ For this reason, a lot of research work is carried out on secondary metabolites of marine invertebrates associated microorganisms that were regarded as the real producers of their host-derived compounds.¹²⁾ During our search for structurally novel and bioactive natural products from fungus isolated from unusual or specialized ecological niches. We have investigated sponge-associated fungal strains for discovery new bioactive natural product from marine invertebrates distributed widely surrounding Jeju-Island. The fungal strain *Aspergillus versicolor* (F12L0104MEA) was isolated from the sponge *Callyspongia confederata* collected from Jeju-Island in Korea.

Fungi belonging to *Aspergillus* species have been proven to be prolific sources of structurally intriguing secondary metabolites of fungal origin, such as

11) Gerwick, W. H.; Moore, B. S., *Chem. Biol.* **2012**, 19, 85-98

12) Zhuang, Y.; Teng, X.; Wang, Y.; Liu, P.; Li, G.; Zhu, W. *Organic Letters* **2011**, 13, 5, 1130-1133

alkaloids, peptides, polyketides, and terpenes, and these compounds exhibited antimicrobial activity and cytotoxicity.¹³⁾ The extract of the fermentation broth of *Aspergillus versicolor* showed significant anti-inflammatory activity by inhibiting the production of PGD₂, LTC₄. Bioassay-guided fractionation yielded four known sesquiterpenoids and diketopiperazines. We described the isolation, structure elucidation and biological evaluation of the compounds.

13) Ji, N.-Y.; Liu, X.-H.; Miao, F.-P.; Qiao, M.-F. *Organic Letters* **2013**, 15, 10, 2327-2329

3.2 Result and Discussion

3.2.1 Structural Elucidation

3.2.1.1 Sydonic acid (**3**)

F12L0104_B4_B (**3**) was obtained as a colorless amorphous solid. The molecular formula of **3** was determined as C₁₅H₂₂O₄, requiring five degrees of unsaturation. The ¹H NMR spectrum showed a downfield doublet proton H-5 (δ_H 7.11, *J* = 8.1 Hz), H-4 (δ_H 7.56, dd, *J* = 8.1, 1.6 Hz) and H-2 (δ_H 7.59, d, *J* = 1.6 Hz) indicated that 1,3,4-trisubstituted benzene ring moiety. Three bond HMBC correlation from H-5 (δ_H 7.11, *J* = 8.1 Hz) to C-1 (δ_C 156.1) and carbon chemical shift for C-1 indicate hydroxy group should be attached at C-1.¹⁴⁾ The analysis of the ¹H, 2D NMR data and comparison with literature data suggested that compound **3** is a previously reported sesquiterpenoids, sydonic acid (**3**).¹⁵⁾

14) Liu, J.; Li, F.; Kin, E. L.; Li, J. J.; Hong, J.; Bae, K. S.; Chung, H. Y.; Kim, H. S.; Jung, J. H. *J. Nat. Prod.* **2011**, 74, 1826-1829

15) Hamasaki, T.; Nagayama, K.; Hatsuda, Y. *Agric. Biol. Chem.* **1978**, 42, 37-40

Table 3.1 Physical and spectral properties of sydonic acid (**3**)

				Colorless amorphous solid
				Molecular formula : C ₁₅ H ₂₂ O ₄
				LRMS : <i>m/z</i> 266.1
				UV (MeOH) λ _{max} : 210, 240, 300 nm
				[α] _D ²⁵ : 0 (c 0.40, CHCl ₃)

NMR Data^a

No.	δ _C , m ^b	δ _H , m, J(Hz)	COSY	HMBC
1	156.1, C			
2	119.2, CH	7.59 (d, 1.6)		4, 6, 15
3	129.6, C			
4	121.1, CH	7.56 (dd, 8.1, 1.6)	5	2, 6, 15
5	126.2, CH	7.11 (d, 8.1)	4	1, 3, 7
6	135.1, C			
7	79.3, C			
8	42.7, CH ₂	1.82 (m) 1.94 (m)	9	
9	21.6, CH ₂	1.32 (m)	8, 10	
10	38.8, CH ₂	1.17 (m)	9, 11	
11	27.7, CH	1.52 (m)	10, 12, 13	
12	22.4, CH ₃	0.85 (d, 6.6)	11	
13	22.4, CH ₃	0.85 (d, 6.6)	11	
14	28.9, CH ₃	1.70 (s)		6, 7, 8
15	171.0, C			

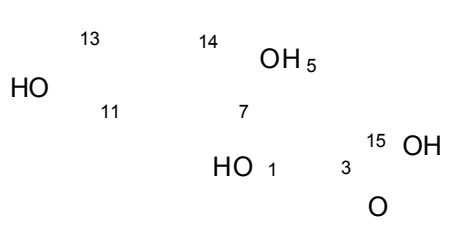
^a 700 MHz for ¹H NMR and 125MHz for ¹³C NMR. ^b The numbers of attached protons were determined by analysis of 1D and 2D spectroscopic data.

3.2.1.2 12-hydroxysydonic acid (**4**)

F12L0104_B6_B (**4**) was obtained as a colorless amorphous solid that analyzed for the molecular formula $C_{15}H_{22}O_4$ based on 1H and 2D NMR data. 1D and 2D NMR spectrums and UV data are similar with compound **3**. The difference was observed at alkyl chain part. The 1H NMR spectrum of **4** indicated one doublet methyl proton H-13 (δ_H 0.77, d, $J = 6.7$ Hz), while that of **3** was two doublet methyl protons. In addition, the chemical shift of H-12 (δ_H 3.20, dd, $J = 10.5, 6.4$ Hz, δ_H 3.29, m), C-12 (δ_C 66.8) indicated that compound **4** had a hydroxy group at C-12. The analysis of the 1H , 2D NMR data and comparison with literature data indicated that compound **4** is a previously reported sesquiterpenoids, 12-hydroxysydonic acid (**4**).¹⁶⁾

16) Chung, Y-M.; Wei, C-K.; Chuang, D-W.; El-Shazly, M.; Hsieh, C-T.; Asai, T.; Oshima, Y.; Hsieh, T-J.; Hwang, T-L.; Wu, Y-C.; Chang, F-R. *Bioorg. Med. Chem.* **2013**, 21, 3866-3872

Table 3.2 Physical and spectral properties of 12-hydroxysydonic acid (**4**)

	Colorless amorphous solid
	Molecular formula : C ₁₅ H ₂₂ O ₄
	LRMS : <i>m/z</i> 282.72
	UV (MeOH) λ _{max} : 210, 240, 300 nm
	[α] ²⁵ _D : 0 (c 0.40, CHCl ₃)

NMR Data^a

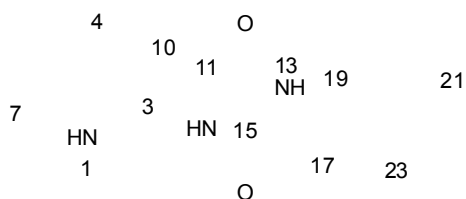
No.	δ _C , m ^b	δ _H , m, J(Hz)	COSY	HMBC
1	155.3, C			
2	117.1, CH	7.30 (d, 1.3)		4, 6, 15
3	130.0, C			
4	120.0, CH	7.39 (dd, 8.0, 1.3)	5	2, 6
5	126.2, CH	7.22 (dd 8.0, 2.3)	4	1, 3, 7
6	136.5, C			
7	76.2, C			
8	42.2, CH ₂	1.73 (m)		
		1.93 (m)		
9	20.5, CH ₂	1.19 (m)	10	
		1.37 (m)		
10	33.1, CH ₂	0.97 (m)	9, 11	
		1.30 (m)		
11	35.4, CH	1.45 (m)	10, 12, 13	
12	66.8, CH ₂	3.20 (dd, 10.5, 6.4)	11	
		3.29 (m)		
13	15.6, CH ₃	0.77 (d, 6.7)	11	10, 11, 12
14	27.2, CH ₃	1.54 (s)		6, 7, 8
15	168.2, C			

^a 700 MHz for ¹H NMR and 125MHz for ¹³C NMR. ^b The numbers of attached protons were determined by analysis of 1D and 2D spectroscopic data.

3.2.1.3 Cyclo(Trptophyl-Phenylalanyl) (**5**)

F12L0104_B4_A (**5**) was obtained as a white amorphous solid. Its molecular formula, $C_{20}H_{19}N_3O_2$, was determined on the basis of the LRMS m/z 333.3 $[M]^+$ and this was supported by 1H and 2D NMR, requiring 13 degree of unsaturation. 1H and 2D NMR spectra of compound **5** showed the presence of two amide groups. The analysis of 1H , 2D NMR compare of literature data identified that compound **5** is a previously reported diketopiperazine, cyclo(Trp-Phe) (**5**).¹⁷⁾

17) Chu, D.; Peng, C.; Ding, B.; Liu, F.; Zhang, F.; Liu, H.; Li, Z. *Bioprocess Biosyst. Eng.* **2011**, 223-229

Table 3.3 Physical and spectral properties of cyclo(Trp-Phe) (**5**)

White amorphous solid

Molecular formula : C₂₀H₁₉N₃O₂**LRMS** : *m/z* 333.3**UV** λ_{max} : 200, 220, 280 nm**NMR Data**

No.	Data from literature ^a		Data from experiment ^b	
	δ _C , m ^b	δ _H , m, J(Hz)	δ _C , m ^b	δ _H , m, J(Hz)
1	-	10.89 (s)		10.92 (s)
2	120.8, CH	6.96 (d, 2.20)	124.8	6.99 (d, 2.20)
3	108.8, C	-	109.2	
4	118.4, CH	7.48 (dd, 7.17, 1.00)	119.2	7.50 (d, 7.62)
5	118.7, CH	6.98 (ddd, 7.17, 7.45, 1.45)	118.9	7.00 (ddd, 7.96, 6.84, 0.93)
6	124.4, CH	7.08 (ddd, 7.73, 7.45, 1.00)	121.3	7.09 (ddd, 8.21, 6.91, 1.06)
7	111.3, CH	7.32 (dd, 7.73, 7.45)	111.8	7.34 (d, 7.84)
8	136.0, C		136.5	
9	127.5, C		127.9	
10	29.6, CH ₂	2.52 (dd, 14.47, 5.68) 2.81 (dd, 14.47, 4.46)	30.1	2.54 (dd, 14.61, 5.87) 2.81 (dd, 14.53, 4.48)
11	55.2, CH	3.98 (m)	55.7	3.99 (m)
12	166.2		167.1	
13	-	7.91 (d, 2.00)		7.96 (d, 2.04)
14	55.6, CH	3.89 (m)	56.1	3.86 (m)
15	166.8		166.7	
16	-	7.71 (d, 2.00)		7.75 (d, 2.22)
17	39.8, CH ₂	1.85 (dd, 13.49, 7.02) 2.45 (dd, 13.49, 4.70)	40.2	1.84 (dd, 13.48, 7.20) 2.45 (dd, 13.55, 4.80)
18	136.5, CH		136.9	
19	128.0, CH	7.16 (m)	128.3	7.16 (m)
20	129.7, CH	6.71 (m)	128.5	6.71 (m)
21	126.3, CH	7.17 (m)	126.9	7.17 (m)
22	129.7, CH	6.71 (m)	128.5	6.71 (m)
23	128.0, CH	7.16 (m)	128.3	7.16 (m)

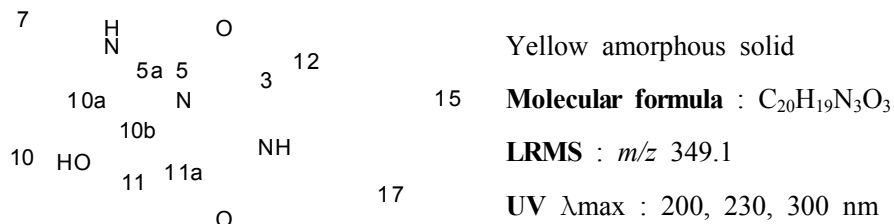
^a Taken in DMSO-d₆, ^b 700 MHz for ¹H NMR and 125MHz for ¹³C NMR. Taken in DMSO-d₆.

3.2.1.4 F12L0104_B3_B (6)

F12L0104_B3_B (6) was isolated as a yellow amorphous solid having the molecular formula of $C_{20}H_{19}N_3O_3$ as determined by LRMS m/z 350.1 $[M+H]^+$ and 1H NMR, requiring 13 degree of unsaturation. Compound **6** was confirmed by spectral of the 1H , 2D NMR data and comparison with literature data, which indicated that is a previously reported photo-oxidation product of ditryptophenaline (**6**).¹⁸⁾

18) Maes, C.; Potgieter, M.; Steyn, P. *J. Chem. Soc. Perkin Trans. I* **1986**, 861-866

Table 3.4 Physical and spectral properties of F12L0104_B3_B (6)



NMR Data

Data from literature ^a			Data from experiment ^b	
No.	δ _C , m ^b	δ _H , m, J(Hz)	δ _C , m ^b	δ _H , m, J(Hz)
1	169.5, C		168.8	
2	-	7.92 (brs)		5.67 (s)
3	55.5, CH	4.45 (t, 5.5)	56.1	4.31 (dd, 10.73, 2.37)
4	167.1, C		166.8	
5	-			
5a	83.9	5.27	85.1	5.52 (s)
6	-	6.62 (d, 3.7)		6.62 (d)
6a	148.2, C		135.6	
7	109.6, CH	6.54 (d, 7.8)	110.5	6.65 (d, 7.93)
8	128.8, CH	7.03 (tt, 7.7, 1.2)	129.3	7.35 (t, 7.55)
9	117.6, CH	6.64 (t, 7.3)	119.8	6.83 (t, 7.33)
10	122.3, CH	7.15 (d, 6.9.)	127.8	7.29 (d, 7.40)
10a	130.9, C		128.4	
10b	85.7		87.0	
11	41.3, CH ₂	1.73 (dd, 13.3, 11.2) 2.40 (dd, 13.1, 6.6)	40.9	2.34 (dd, 13.53, 11.03) 2.75 (dd, 13.6, 6.80)
11a	58.3, CH	4.60 (dd, 11.3, 6.6)	59.0	4.62 (dd, 10.30, 6.67)
12	34.5, CH ₂	3.04 (dd, 14.5, 5.3) 3.13 (dd, 14.5, 5.3)	36.6	2.79 (dd, 14.42, 10.92) 3.65 (dd, 14.40, 3.55)
13	137.2, C		147.4	
14	129.6, CH	7.33 (d, 7.7)	129.0	7.23 (d, 7.31)
15	127.8, CH	7.21 (t, 7.6)	123.0	7.30 (t, 6.86)
16	126.1, CH	7.14 (t, 6.4)	130.6	7.20 (td, 7.65, 0.96)
17	127.8, CH	7.21 (t, 7.6)	123.0	7.30 (t, 6.86)
18	129.6, CH	7.33 (d, 7.7)	129.0	7.23 (d, 7.31)

^a Taken in DMSO-d₆, ^b 700 MHz for ¹H NMR and 125MHz for ¹³C NMR. Taken in CD₃OD.

3.2.2 Anti-inflammatory Activity of compound 3 and 5

The extract from fermentation broth of *Aspergillus versicolor* (F12L010MEA) exhibited significant anti-inflammatory activity by inhibiting the production of PGD_2 , LTC_4 , as shown in Figure 3.1.

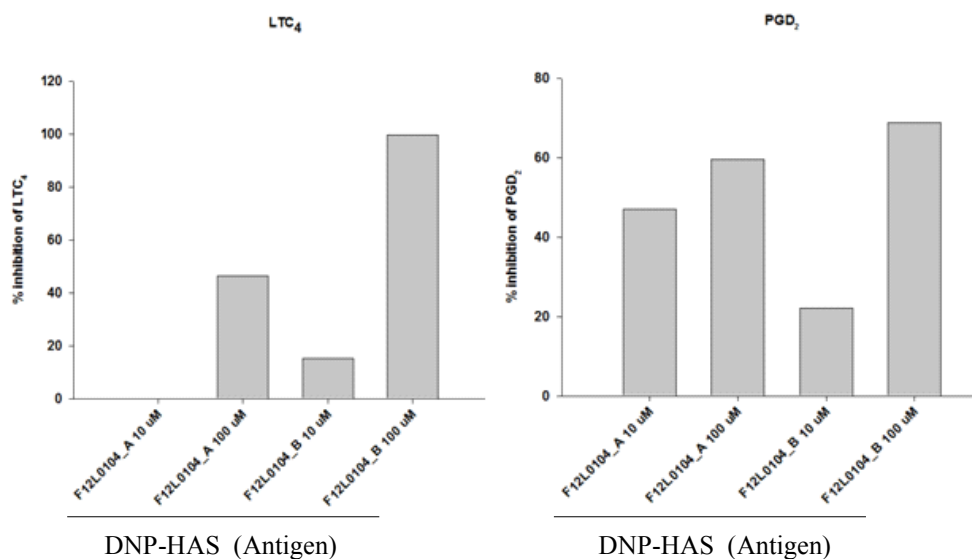


Figure 3.1 Results of Anti-inflammatory activity test

3.3 Experimental

3.3.1 Instruments and Data Collection

All NMR spectra were recorded on a Bruker Advance 700MHz spectrometer with CDCl₃. Low resolution LC-MS data were recorded on an Agilent Technologies 6120 quadrupole LC/MS system with a reversed-phase C₁₈ column (Phenomenex luna 5 μ (2), 4.6 mm X 50 mm, 5 μ m) at a flow rate of 1.0 mL/min. The optical rotation was obtained using a 5.0 cm cell on a Rudolph Research Autopol III. Isolation of pure compounds by HPLC WATERS 1525 binary HPLC pump, WATERS 2489 UV/visible detector was carried out using reversed-phase semipreparative C₁₈ column (Phenomenex luna 5 μ (2), 250 X 10 mm, 5 μ m). Solvent used in partitioning were first grade products of Dae-Jung & Metals Co., Korea. HPLC grade solvent from Burdick & Jackson were used in TLC and HPLC. NMR solvents were purchased from Cambridge Isotope Laboratories (CIL), Inc.

3.3.2 Animal and Fungus Material

Aspergillus versicolor (F12L0104MEA) was isolated from a marine sponge *Callyspongia confederata*. The marine sponge was collected in Jeju-island, Korea at depth of 15-20 m using SCUBA in November 2012. Under sterile conditions, the sponge was rinsed in filtered seawater followed by successive surface sterilization in 70 % EtOH. A piece of tissue from the inner part of the surface sterile sponge was cut and inoculated on malt extract agar (MEA) media plate consisting filtered seawater. The agar plate was transported to the laboratory and incubated at 25 °C. Pure fungal strain was isolated after repeated inoculation on fresh potato dextrose agar (PDA) media plates. The fungus F12L0104MEA was identified as *Aspergillus versicolor* on the basis of sequence analysis of the ribosomal internal transcribed spacer (ITS) and showed 93 % similarity to *Aspergillus versicolor* (EU 586040)

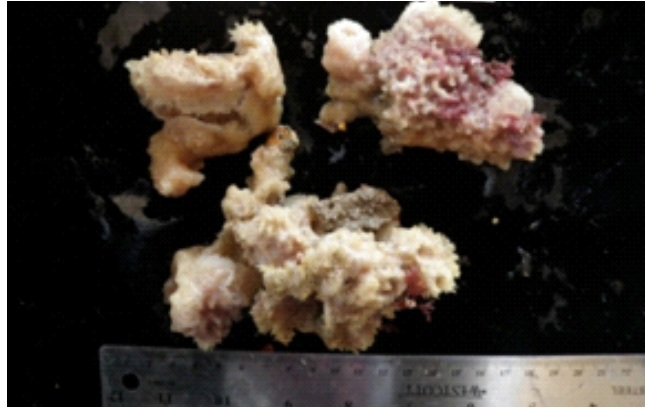


Figure 3.2 Picture of the sponge, *Callyspongia confoederata*



Figure 3.3 Picture of the marine-derived fungus, *Aspergillus versicolor*

3.3.3 Cultivation, Extraction and Isolation

The fungus was cultured in potato dextrose broth (PDB, Difco, Detroit, MI, USA) using filtered seawater. The fungus was inoculated with cultured seed agar blocks into 4 L culture Pyrex flasks each containing 1 L of PDB and cultured at 27 °C with shaking at 130 rpm. After 15 days, Fungal cells were separated from broth (32 L) by gauze filter. The fungal cells were extracted with methanol and acetone (1:1) and filtered. The filtered culture broth was extracted two times with EtOAc and evaporated to yield 2.3 g of crude extract. The crude extract was subjected to silica gel open column chromatography using step-gradient solvent system of 0-100% CHCl₃-MeOH to afford 7 fractions. Fraction 4 (230.7 mg) was purified by C₁₈ reversed-phase HPLC (Phenomenex luna 5 μ (2), 250 X 10 mm, 5 μ m, 2.5 ml/min, UV = 210 nm, 300 nm), eluting with 50 % CH₃CN in H₂O to afford Cyclo(Trp-Phe) (**5**, 2.5 mg) and Sydonic acid (**3**, 2.5 mg). Fraction 6 (261.4 mg) was separated to afford 12-hydroxysydonic acid (**4**, 1.2 mg), eluting with 20 % CH₃CN in H₂O. Fraction 3 (131.4 mg) also was purified using the same method as fraction 4, eluting with 40 % CH₃CN in H₂O to afford F12L0104_B3_B (**6**, 0.8 mg)

3.3.4 Bioassay procedure

A. Culture and Activation of Mouse Bone Marrow Derived Mast Cells (BMMC)

Mouse bone marrow cells derived male BALB/c mice were maintained in RPMI 1640 containing 10 % FBS, 100 µg/mL penicillin, 100 µg/mL streptomycin, and 100 µM MEM Non-essential amino acid solution with 20 % PWM-SCM, as IL-3's supply source, which contains pokeweed mitogen-stimulate spleen cell-conditioned medium for 3 weeks in humidified atmosphere of 5 % CO₂ at 37 °C. Over 90 % homogenous bone marrow-derived mast cells (BMMC) were used for activation test.

B. Measurement of PGD₂ Production

BMMC were sensitized by IgE for 12 hours to measure PGD₂ depended on COX-2 and then was washed. The sensitized BMMC (1X10⁶ cells/mL) were treated with marine natural product extracts for 1 hour, after that additionally treated with DNP-HSA (Antigen) for 7 hours. PGD₂ production in the media was measured by ELISA. During the measurement, PGD₂ production was generated by COX-1 was inhibited by 10 µg/mL Aspirin.

C. Measurement of LTC₄ Production

The marine natural product extracts were added to sensitized BMMC by IgE for 1 hour before the measurement of LTC₄ production. The sensitized BMMC by DNP-HSA was further incubated for 15 minutes, the LTC₄ production of the supernatant was measured by ELISA.

D. Measurement of Degranulation

Sensitized BMMC by IgE for 1 hour was treated with marine natural product extracts before measurement of degranulation. Furthermore, Ag or SCF added to

treated BMMC which was incubated for 15 minutes, the β -hexosaminidase (degranulation indicator) in the supernatant was measured by colorimetry.

Chapter 4

Sterol Derivatives from a Marine Sponge *Homaxinella* sp.

4.1 Introduction

Marine sponges of the genus *Homaxinella* are reported to contain prolific sources of marine natural products. In continuation of our search for bioactive metabolites from a marine sponges, crude extract of the sponge *Homaxinella* sp. collected from South Sea of Korea showed we noticed significant antibacterial activity against selectively targeted Gram-positive strain (*staphylococcus aureus* CCARM 0205). Three Sterol derivatives were isolated from bioactivity-guided fractionation from MeOH extract. In the study, we described the isolation and structural elucidation of three known compounds.

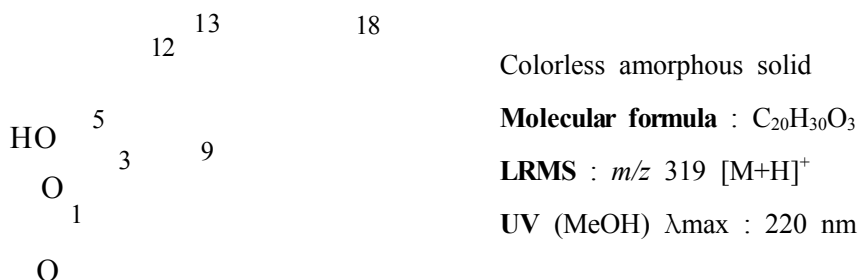
4.2 Result and Discussion

4.2.1 Structural Elucidation

4.2.1.1 Demethylincisterol A₁ (7)

Compound **7** was isolated as a white solid. Its molecular formula was determined as C₂₀H₃₀O₃ based on LRMS in at m/z 319 [M+H]⁺ and ¹H NMR, requiring 6 degree of unsaturation. The ¹H NMR spectra of **7** showed one singlet methyl group, three doublet methyl groups, one disubstitued alkene group. Compound **7** was identified as previously reported demethylincisterol A₁ by comparison of their respective spectral data (¹H NMR, LRMS) with AntiMarin database and literature data.¹⁹⁾

19) Mansoor, T. A.; Hong, J.; Lee, C. -O.; Bae, S. -J.; Im, K. S.; Jung, J. H. *J. Nat. Prod.* **2005**, 68, 331-336

Table 4.1 Spectral properties of demethylincisterol A₁ (7)**NMR Data**

No.	Data from literature ^a		Data from experiment ^b
	δ _C , m ^b	δ _H , m, J(Hz)	δ _H , m, J(Hz)
1	173.6, C		
2	112.6, CH	5.67 (d, 1.8)	5.66 (d, 1.4)
3	172.9, C		
4	104.1, C		
5	36.2, CH ₂	2.23 (ddd, 14.0, 4.0, 2.5)	2.30 (ddd, 14.0, 3.7, 2.3)
6	36.5, CH ₂	1.98 (ddd, 13.0, 5.0, 2.5) 1.62 (m)	2.00 (ddd, 12.9, 4.2, 2.2) 1.60 (m)
7	49.0, C		
8	51.7, CH	2.66 (ddd, 12.0, 7.0, 1.8)	2.67 (ddd, 12.1, 6.7, 1.5)
9	22.3, CH ₂	1.73 (m) 1.62 (m)	1.75 (m) 1.60 (m)
10	30.2, CH ₂	1.92 (m) 1.49 (m)	1.95 (m) 1.49 (m)
11	56.7, CH	1.50 (m)	1.51 (m)
12	12.1, CH ₃	0.68 (s)	0.63 (s)
13	41.5, CH	2.08 (m)	2.00 (m)
14	21.4, CH ₃	1.05 (d, 7.0)	1.05 (d, 6.6)
15	138.6, CH	5.24 (dd, 15.0, 9.5)	5.24 (dd, 15.7, 8.7)
16	128.3, CH	5.36 (dt, 15.0, 8.5)	5.36 (dt, 15.2, 7.0)
17	43.1, CH ₂	1.85 (m)	1.87 (m)
18	29.7, CH	1.57 (m)	1.55 (m)
19	22.7, CH ₃	0.84 (d, 7.0)	0.89 (d, 6.7)
20	22.7, CH ₃	0.88 (d, 7.0)	0.89 (d, 6.7)

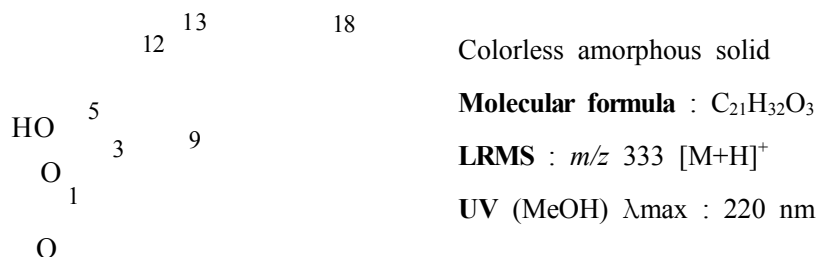
^a Taken in CD₃OD, ^b 700 MHz for ¹H NMR taken in CDCl₃.

4.2.1.2 Demethylincisterol A₂ (**8**)

Compound **8**, a white solid, has the molecular formula C₂₁H₃₂O₃ based on LRMS m/z 333 [M+H]⁺ and ¹H NMR, requiring 6 degree of unsaturation. The ¹H NMR spectra of **8** revealed one singlet methyl group, four doublet methyl groups, one disubstituted alkene group. Compound **8** was identified as previously reported demethylincisterol A₂ (**8**) by analysis of ¹H NMR and comparison with AntiMarin database and literature data.²⁰⁾

20) Mansoor, T. A.; Hong, J.; Lee, C. -O.; Bae, S. -J.; Im, K. S.; Jung, J. H. *J. Nat. Prod.* **2005**, 68, 331-336

Table 4.2 Spectral properties of demethylincisterol A₂ (**8**)



NMR Data

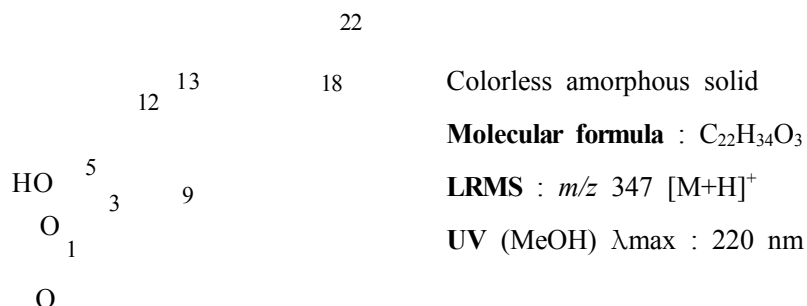
No.	Data from literature ^a		Data from experiment ^b
	δ _C , m ^b	δ _H , m, J(Hz)	δ _H , m, J(Hz)
1	173.6, C		
2	112.6, CH	5.67 (d, 1.8)	5.63 (s)
3	172.9, C		
4	104.1, C		
5	36.2, CH ₂	2.23 (ddd, 14.0, 4.0, 2.5)	2.30 (ddd, 14.0, 3.7, 2.3)
6	36.5, CH ₂	1.98 (ddd, 13.0, 5.0, 2.5) 1.62 (m)	2.00 (ddd, 12.9, 4.2, 2.2) 1.62 (dt, 13.3, 3.6)
7	49.0, C		
8	51.7, CH	2.66 (ddd, 12.0, 7.0, 1.8)	2.64 (dd, 10.9, 7.5)
9	22.3, CH ₂	1.73 (m) 1.62 (m)	1.71 (m) 1.62 (m)
10	30.2, CH ₂	1.92 (m) 1.49 (m)	1.98 (m) 1.46 (m)
11	56.7, CH	1.50 (m)	1.50 (m)
12	12.1, CH ₃	0.68 (s)	0.63 (s)
13	41.5, CH	2.08 (m)	2.00 (m)
14	21.4, CH ₃	1.04 (d, 7.0)	1.03 (d, 6.3)
15	137.5, CH	5.21 (m)	5.15 (dd, 15.1, 8.4)
16	133.2, CH	5.26 (m)	5.22 (dt, 15.1, 7.8)
17	43.1, CH	1.85 (m)	1.86 (m)
18	29.7, CH	1.57 (m)	1.53 (m)
19	22.0, CH ₃	0.85 (d, 7.0)	0.82 (d, 6.9)
20	21.5, CH ₃	0.86 (d, 7.0)	0.84 (d, 6.6)
21	20.0, CH ₃	0.94 (d, 6.5)	0.91 (d, 6.9)

^a Taken in CD₃OD, ^b 700 MHz for ¹H NMR taken in CDCl₃.

4.2.1.3 Demethylincisterol A₄ (9)

Compound **9** was isolated as a white solid. Its molecular formula was determined as C₂₂H₃₄O₃ based on LRMS m/z 347 [M+H]⁺ and ¹H NMR, requiring 6 degree of unsaturation. The ¹H NMR spectra of **9** revealed one singlet methyl group, three doublet methyl groups, one triplet methyl group and one disubstituted alkene group. ¹H NMR data of **9** were almost same as compound **8**. The only difference in the side chain δ_H 0.82 as a triplet methyl in the ¹H NMR spectrum indicated that it has an ethyl group. Using the partial structure of compound **9** as input for dereplication searches with AntiMarin database and literature data indicated that compound **9** is a previously reported demethylincisterol A₄ (**9**).²¹⁾

21) Mansoor, T. A.; Hong, J.; Lee, C. -O.; Bae, S. -J.; Im, K. S.; Jung, J. H. *J. Nat. Prod.* **2005**, 68, 331-336

Table 4.3 Spectral properties of demethylincisterol A₄ (9)**NMR Data**

No.	Data from literature ^a		Data from experiment ^b
	δ _C , m ^b	δ _H , m, J(Hz)	δ _H , m, J(Hz)
1	173.6, C		
2	112.6, CH	5.67 (d, 1.8)	5.66 (d, 1.3)
3	172.9, C		
4	104.1, C		
5	36.2, CH ₂	2.23 (ddd, 14.0, 4.0, 2.5)	2.30 (dt, 14.0, 2.4)
6	36.5, CH ₂	1.98 (ddd, 13.0, 5.0, 2.5)	2.00 (m)
		1.62 (m)	1.59 (m)
7	49.0, C		
8	51.7, CH	2.66 (ddd, 12.0, 7.0, 1.8)	2.67 (dd, 11.0, 6.8)
9	22.3, CH ₂	1.73 (m)	1.75 (m)
		1.62 (m)	1.66 (dt, 13.6, 3.8)
10	30.2, CH ₂	1.92 (m)	1.98 (m)
		1.49 (m)	1.49 (m)
11	56.7, CH	1.50 (m)	1.52 (m)
12	12.1, CH ₃	0.68 (s)	0.63 (s)
13	41.5, CH	2.08 (m)	2.00 (m)
14	21.4, CH ₃	1.07 (d, 7.0)	1.07 (d, 6.6)
15	139.9, CH	5.22 (m)	5.11 (dd, 15.2, 8.7)
16	132.2, CH	5.26 (m)	5.20 (dd, 15.0, 8.6)
17	53.2, CH	1.85 (m)	1.89 (dt, 14.0, 4.6)
18	29.7, CH	1.57 (m)	1.57 (m)
19	21.5, CH ₃	0.82 (d, 6.0)	0.82 (d, 6.3)
20	20.0, CH ₃	0.87 (d, 6.0)	0.87 (d, 6.3)
21	27.2, CH ₂	1.45 (m)	1.45 (m)
		1.22 (m)	1.22 (m)
22	14.0, CH ₃	0.84 (t, 7.5)	0.84 (t, 7.3)

^a Taken in CD₃OD, ^b 700 MHz for ¹H NMR taken in CDCl₃.

4.3 Experimental

4.3.1 Instruments and Data Collection

All NMR spectra were obtained using Bruker Advance 700MHz spectrometers [CDCl_3 (δ_{H} 7.26) was used as an internal standard]. Low resolution LC-MS data were measured using a Agilent Technologies 6120 quadrupole LC/MS system with a reversed-phase C_{18} column (Phenomenex luna 5u (2), 4.6 mm X 50 mm, 5 μm) at a flow rate of 1.0 ml/min. Purification of compounds by HPLC WATERS 1525 binary HPLC pump, WATERS 2489 UV/visible detector was carried out using reversed-phase semipreparative C_{18} column (Phenomenex luna 5u (2), 250 X 10 mm, 5 μm). Solvent used in partitioning were first grade products of Dae-Jung & Metals Co., Korea. HPLC grade solvent from Burdick & Jackson were used in TLC and HPLC. NMR solvents were purchased from Cambridge Isotope Laboratories (CIL), Inc.

4.3.2 Animal Material

The marine sponge was collected in South Sea of Korea at depth of 15-20m using SCUBA. The sponge was immediately transported to the laboratory by packing with dry ice. It was stored at -18°C until processed. The sponge was yellow with a soft and resembles branch of tree. A voucher specimen was deposited at the Laboratory of Marine drug, Seoul National University, Korea.



Figure 4.1 Picture of the sponge, *Homaxinella* sp.

4.3.3 Extraction and Isolation

The frozen sponge (1.5 kg, wet wt.) was lyophilized and the dried specimen (350 g) was extracted three times with 50 % MeOH in CH₂Cl₂ at room temperature. The crude extract (31.5 g) was partitioned between CH₂Cl₂ and H₂O. The CH₂Cl₂ layers was evaporated to dryness in vacuo, and then the residue was partitioned between *n*-Hexane (1.65 g) and 10 % aqueous MeOH (3.25 g). The aqueous MeOH layer was fractionated over sephadex LH-20 column chromatography using 50 % MeOH in CH₂Cl₂ to give twelve fractions (1-12). On the basis of the result of TLC analysis, the fractions were combined. Combined fraction (fraction 8 to fraction 11) (1.1 g) was separated by silica normal-phase vacuum flash chromatography using stepped gradient eluent mixture of Hexane and EtOAc. The fourth fraction was separated by reversed-phase HPLC (Phenomenex luna 5u (2), 250 × 10 mm, 5 μm, 2.5 ml/min, UV = 210 nm), eluting with 70 % CH₃CN in H₂O to afford compound **7** (1.0 mg), compound **8** (1.2 mg) and compound **9** (1.1 mg).

Appendix

Figure S1. ^1H NMR spectrum of Marinopyrone D (1) in CD_3CD	55
Figure S2. ^{13}C NMR spectrum of Marinopyrone D (1) in CD_3CD	56
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Figure S14. HSQC NMR spectrum of 12-hydroxysydonic acid (4) in CD_3CD	68
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Figure S18. ^1H NMR spectrum of Demethylincisterol A ₁ (7) in CDCl_3	72
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Figure S20. ^1H NMR spectrum of Demethylincisterol A ₄ (9) in CD_3CD	74

Figure S1. ^1H NMR spectrum of Marinopyrone D (**1**) in CD_3CD

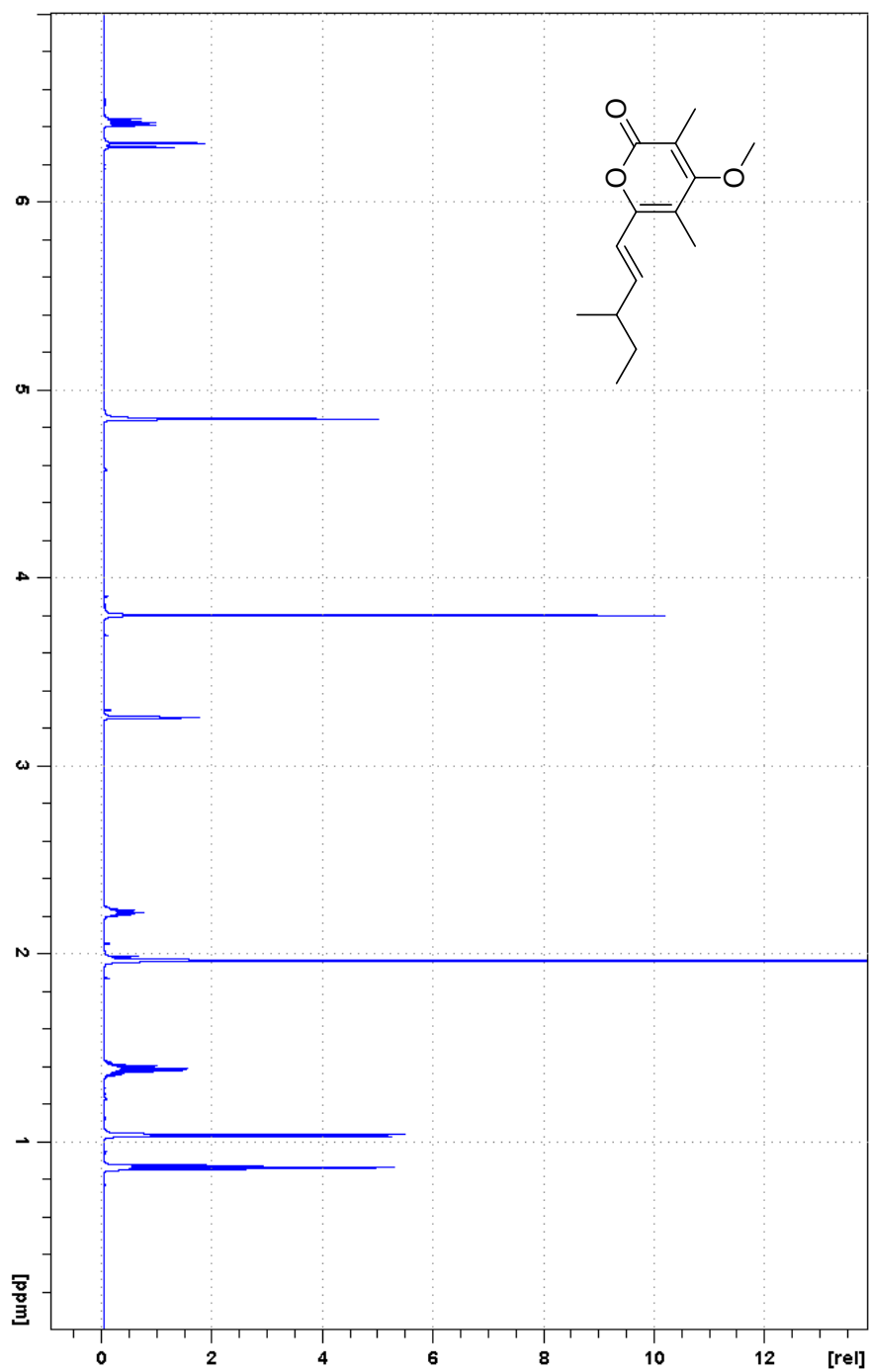


Figure S2. ^{13}C NMR spectrum of Marinopyrone D (**1**) in CD_3CD

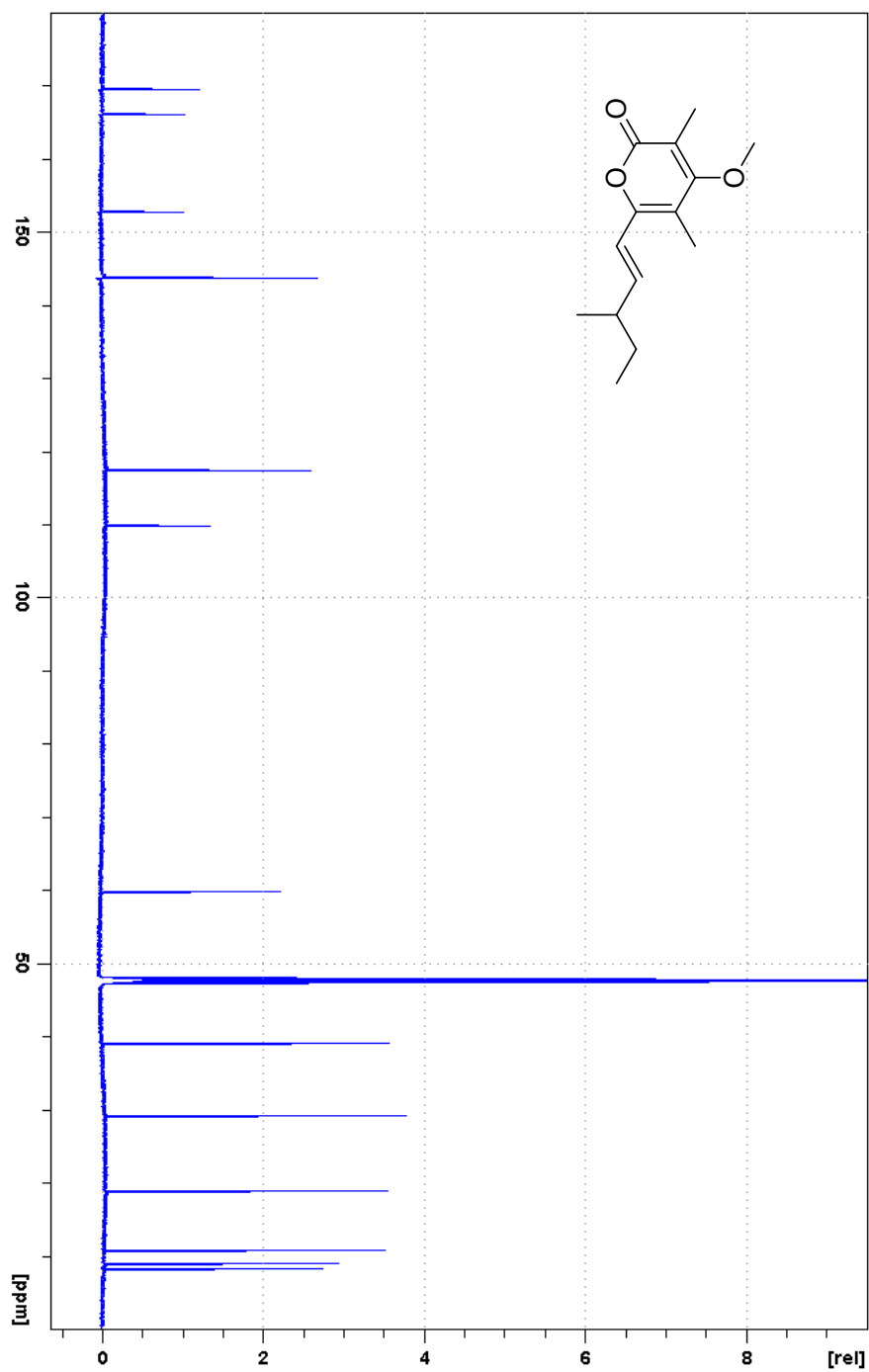


Figure S3. COSY NMR spectrum of Marinopyrone D (**1**) in CD₃CD

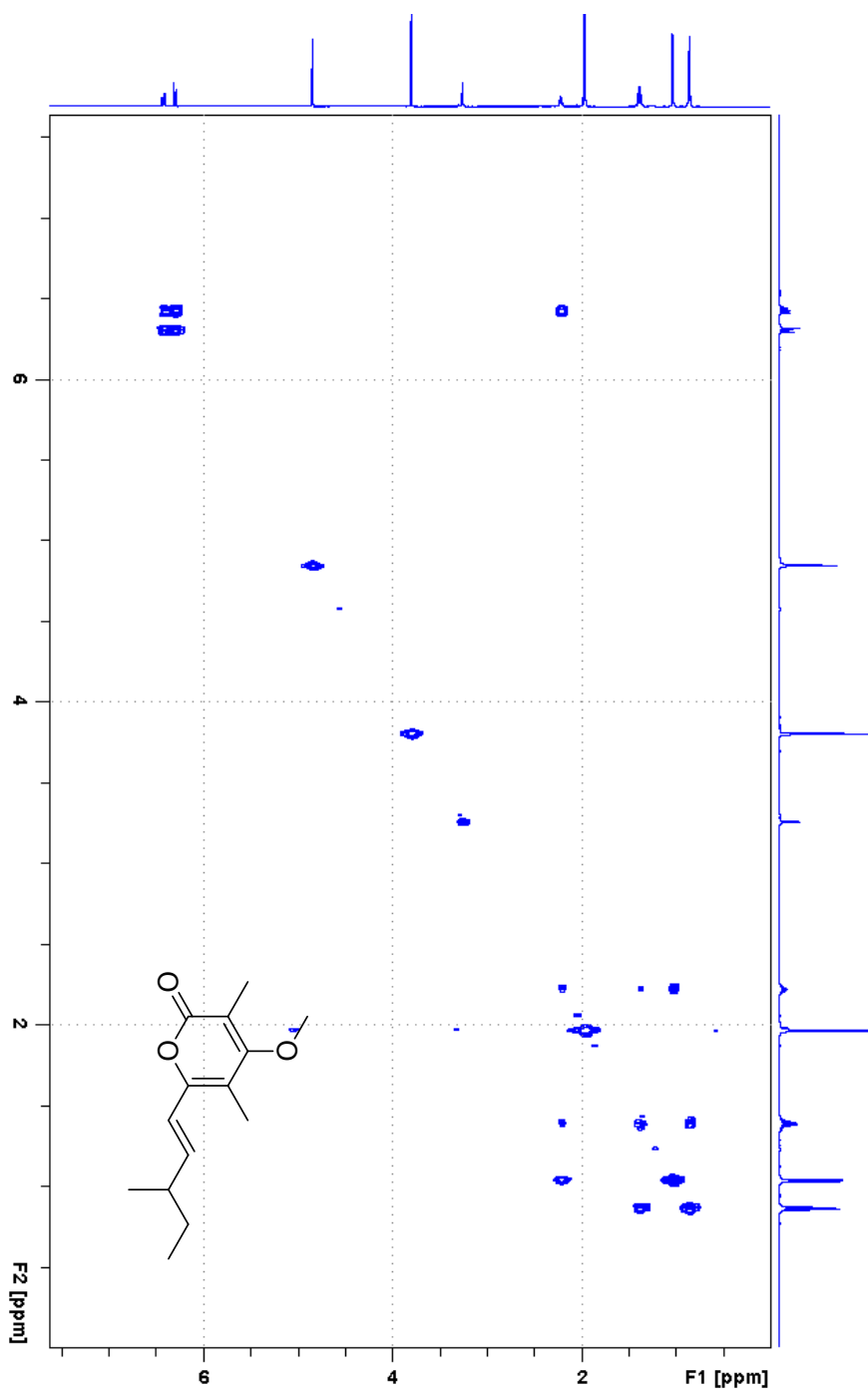


Figure S4. HSQC NMR spectrum of Marinopyrone D (**1**) in CD₃CD

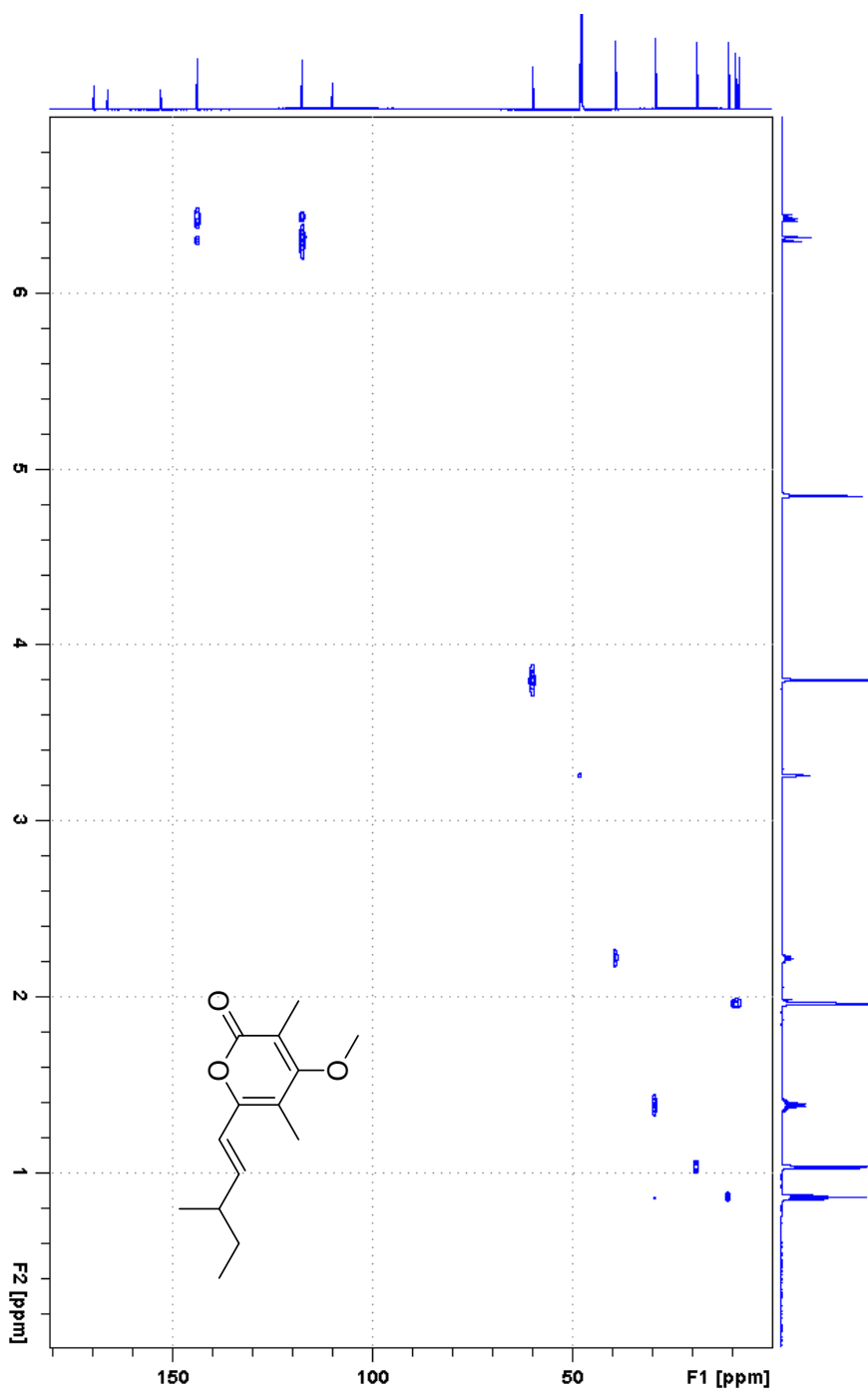


Figure S5. HMBC NMR spectrum of Marinopyrone D (**1**) in CD₃CD

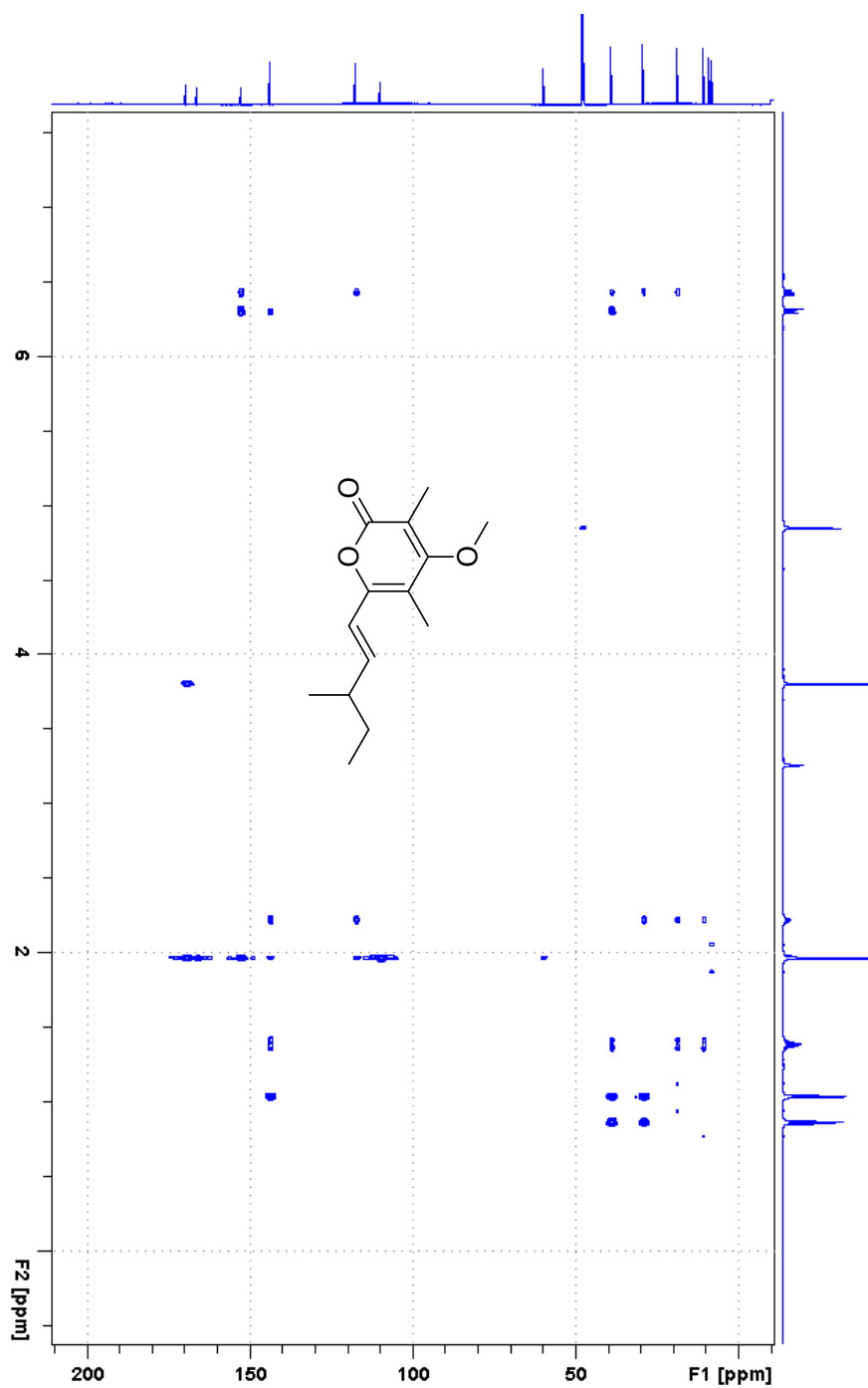


Figure S6. ^1H NMR spectrum of Marinopyrone E (**2**) in CD_3CD

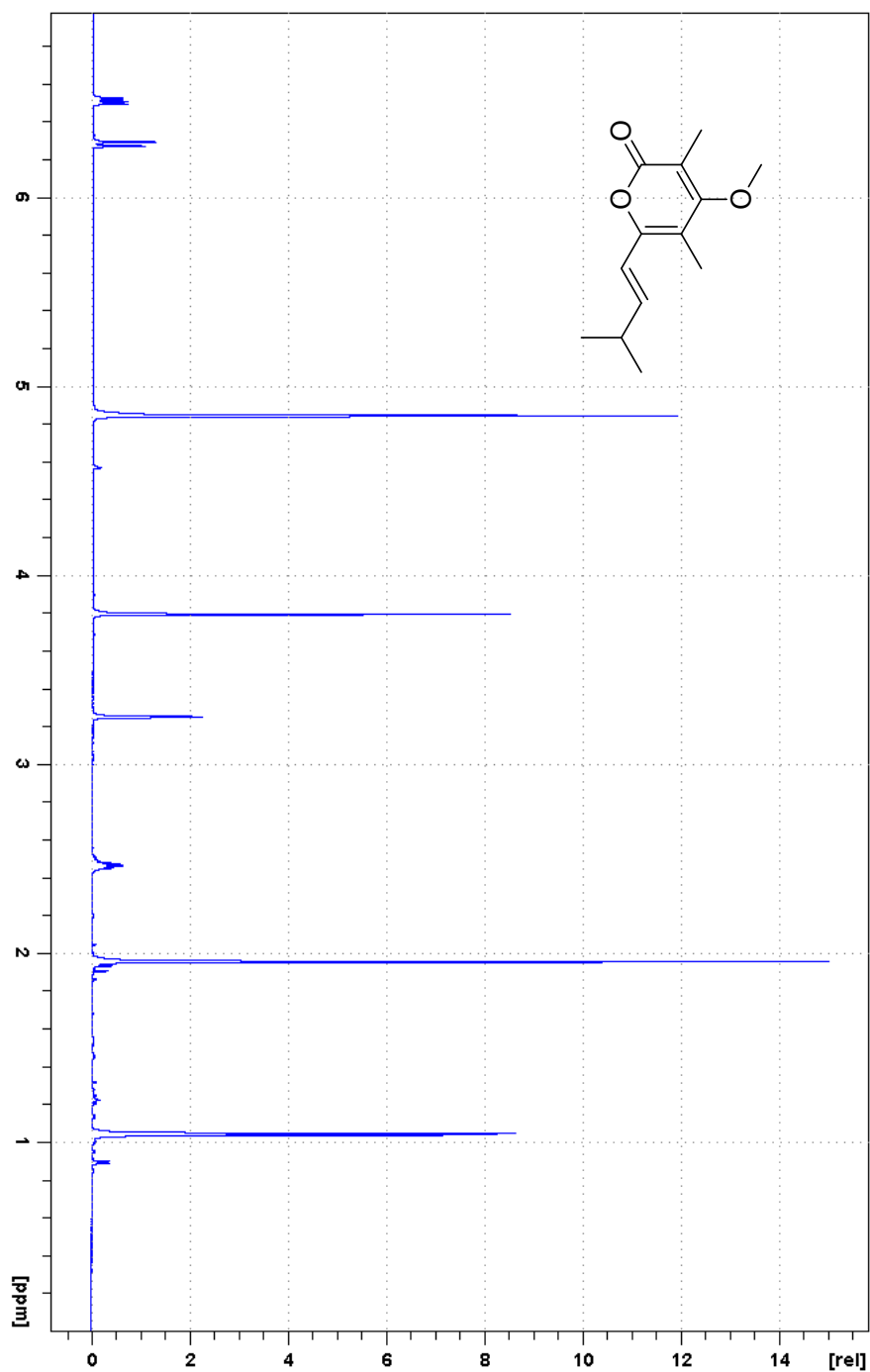


Figure S7. ^1H NMR spectrum of Sydonic acid (**3**) in CDCl_3

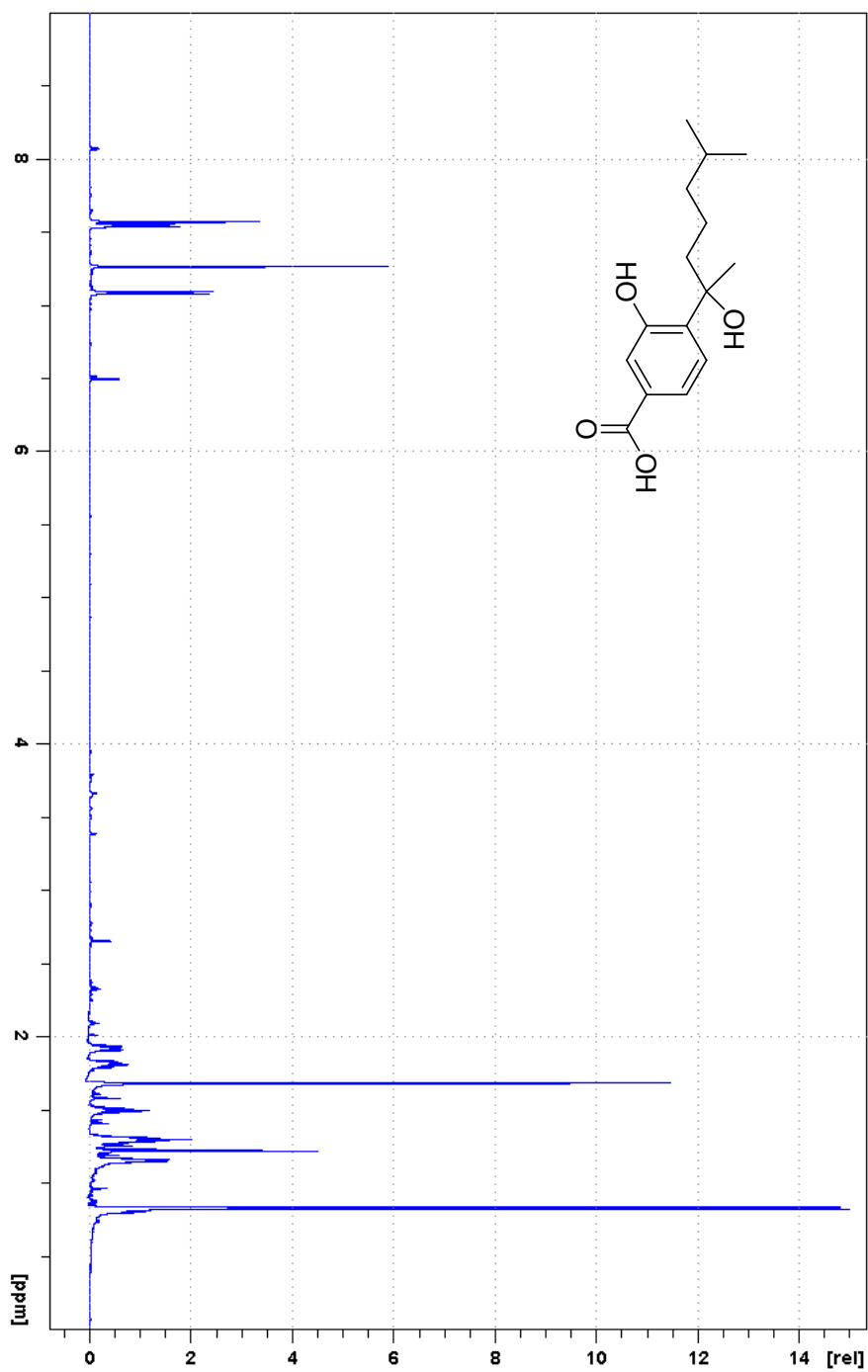


Figure S8. ^{13}C NMR spectrum of Sydonic acid (**3**) in CDCl_3

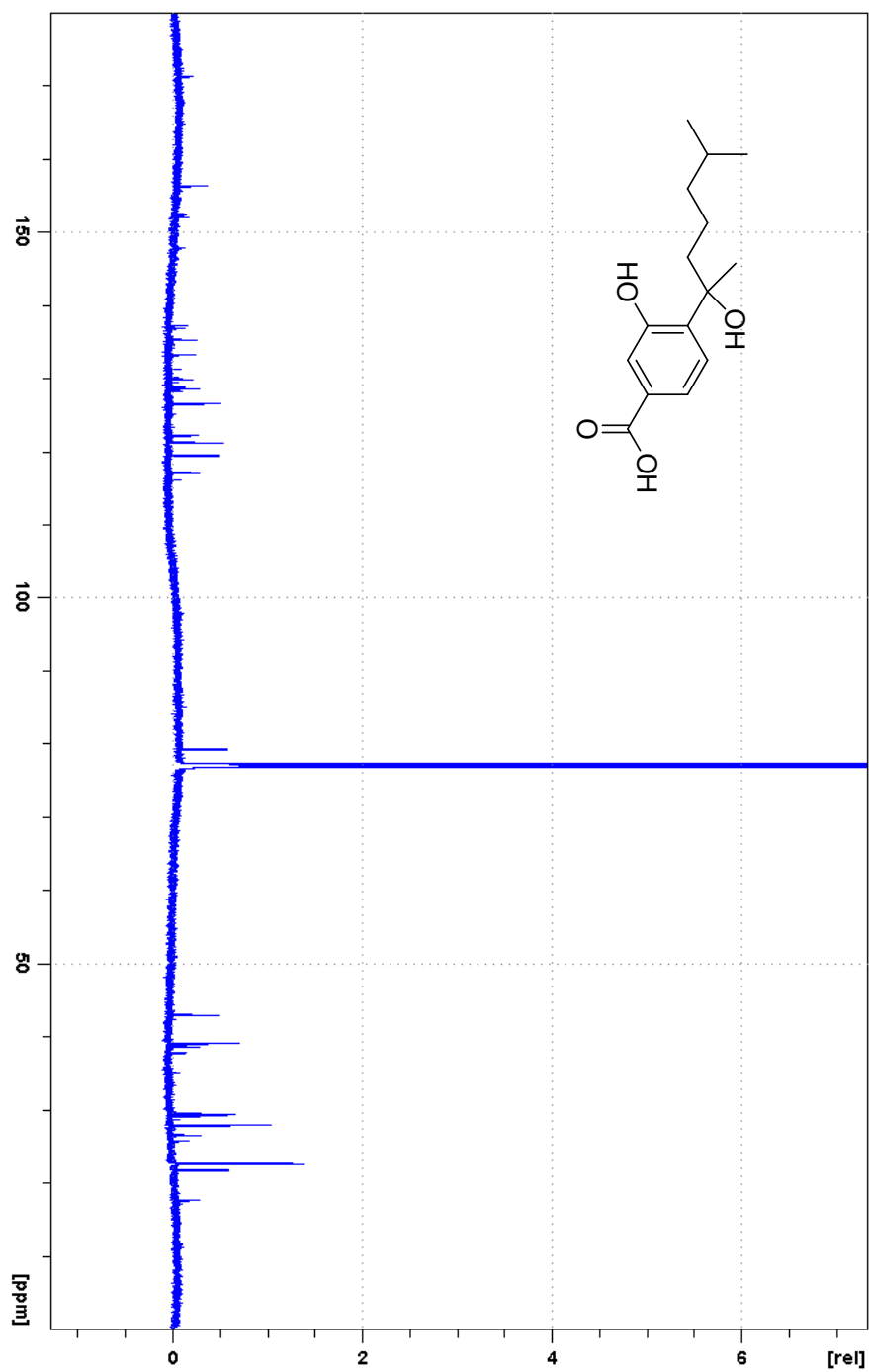


Figure S9. COSY NMR spectrum of Sydonic acid (**3**) in CDCl₃

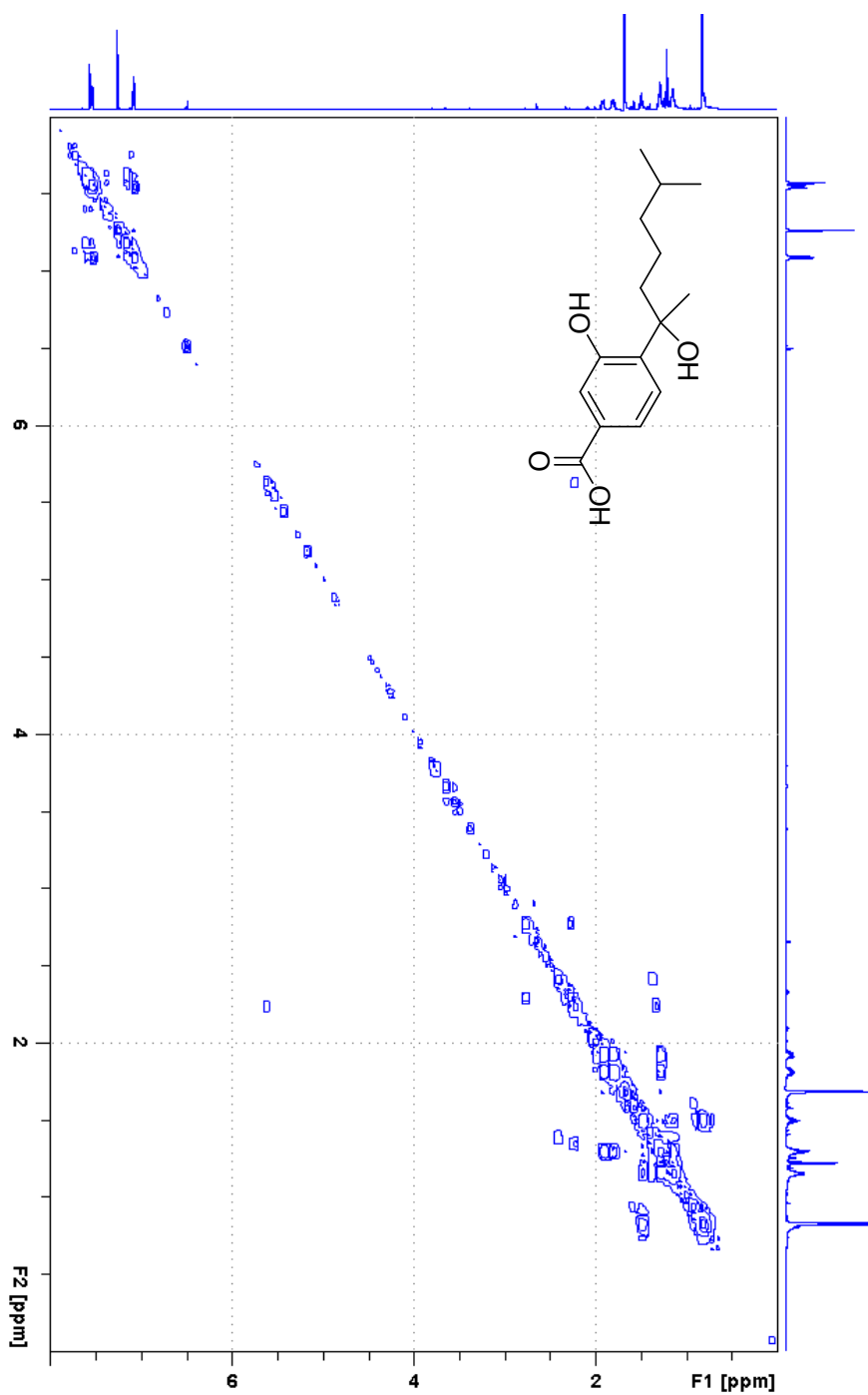


Figure S10. HSQC NMR spectrum of Sydonic acid (**3**) in CDCl₃

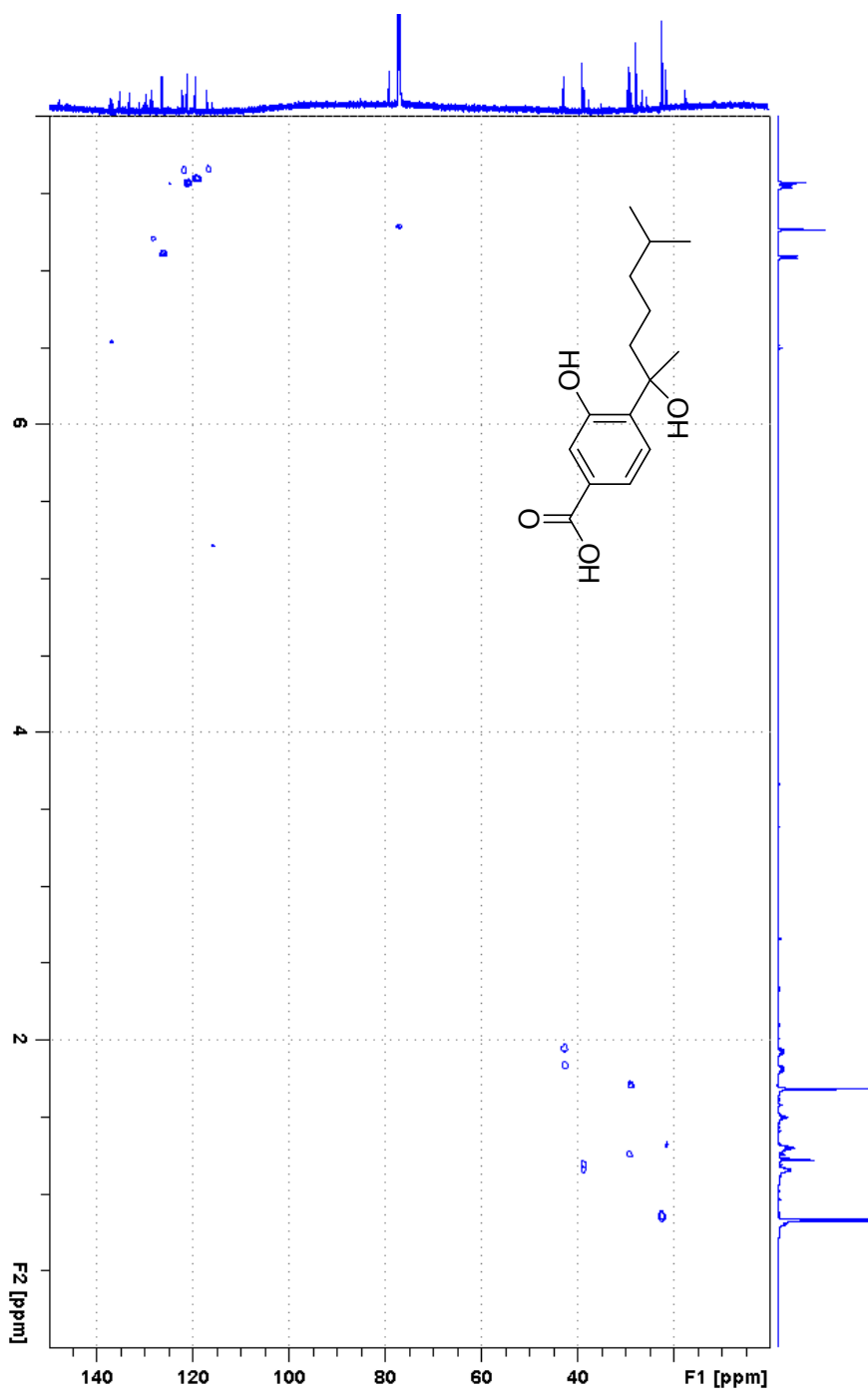


Figure S11. HMBC NMR spectrum of Sydonic acid (**3**) in CDCl₃

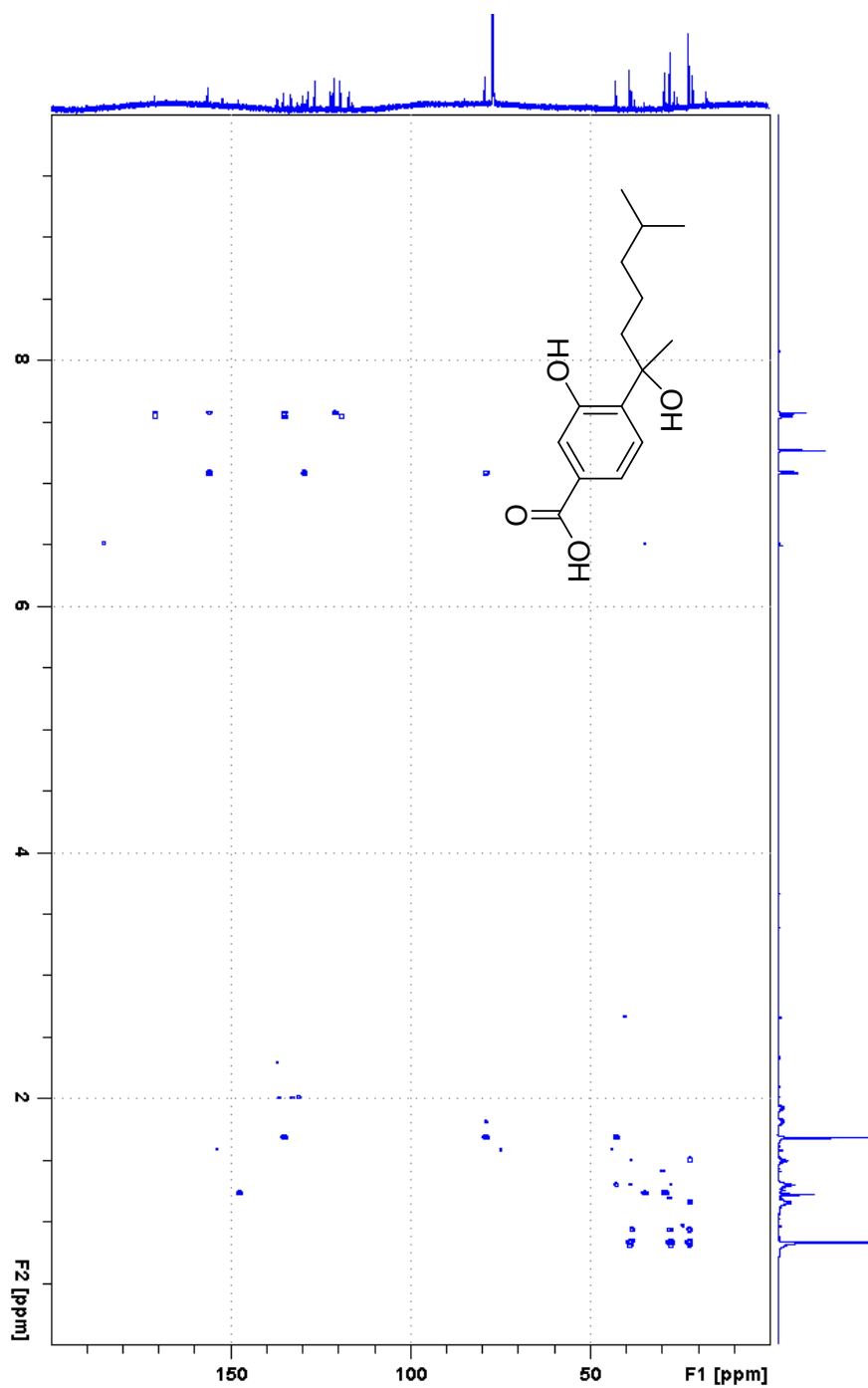


Figure S12. ^1H NMR spectrum of 12-hydroxysydonic acid (**4**) in CD_3CD

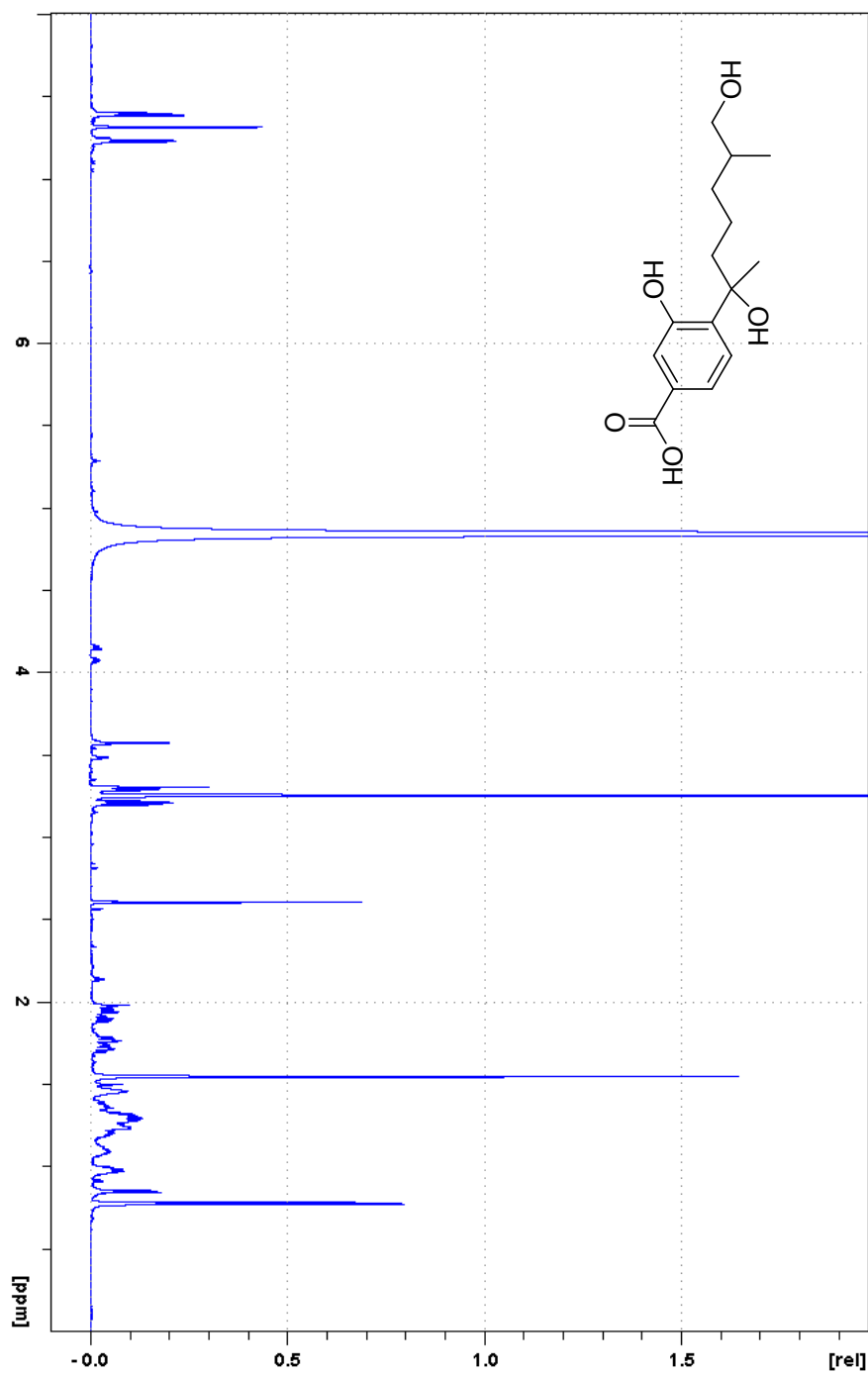


Figure S13. COSY NMR spectrum of 12-hydroxysydonic acid (**4**) in CD₃CD

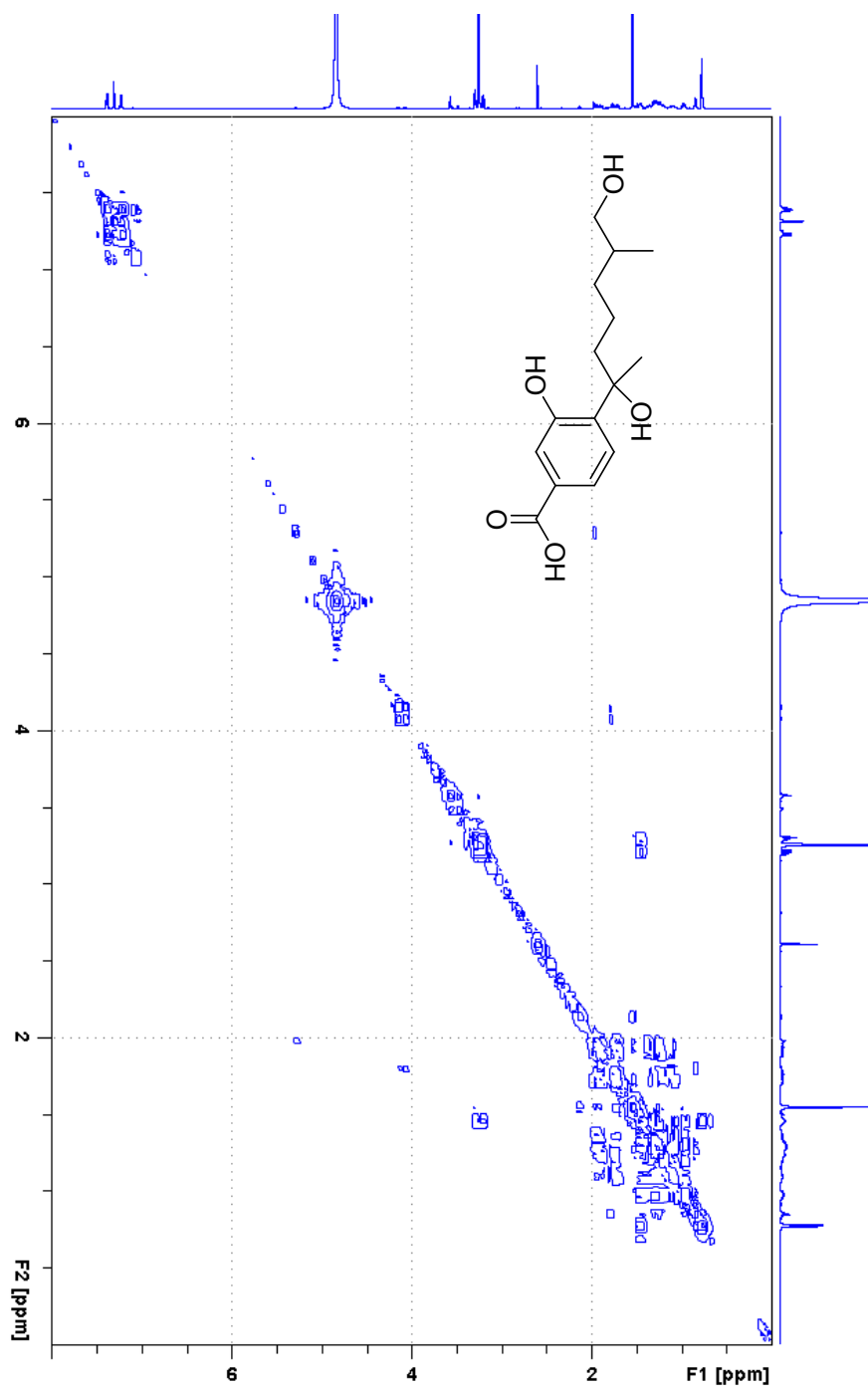


Figure S14. HSQC NMR spectrum of 12-hydroxysydonic acid (**4**) in CD₃CD

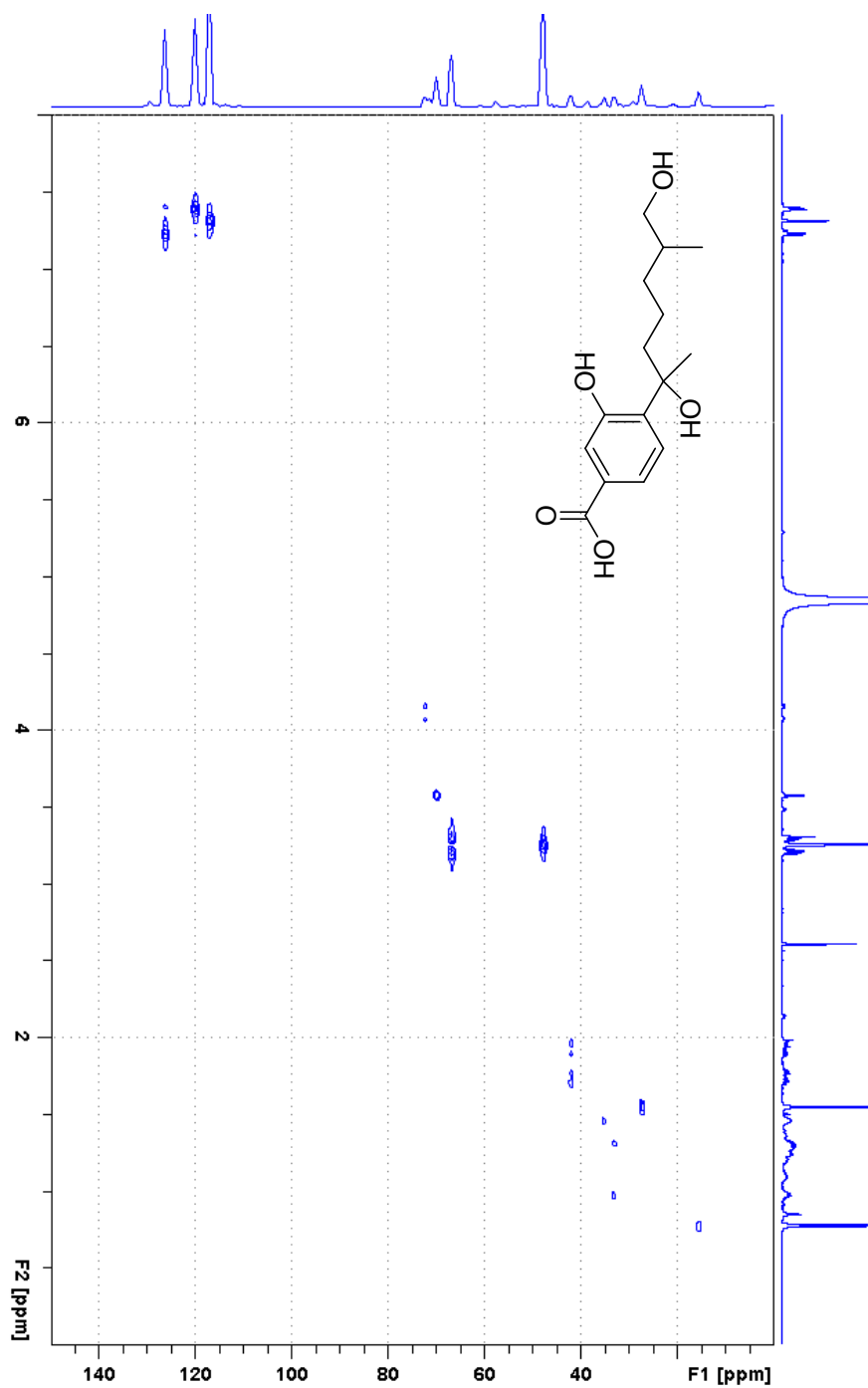


Figure S15. HMBC NMR spectrum of 12-hydroxysydonic acid (**4**) in CD₃CD

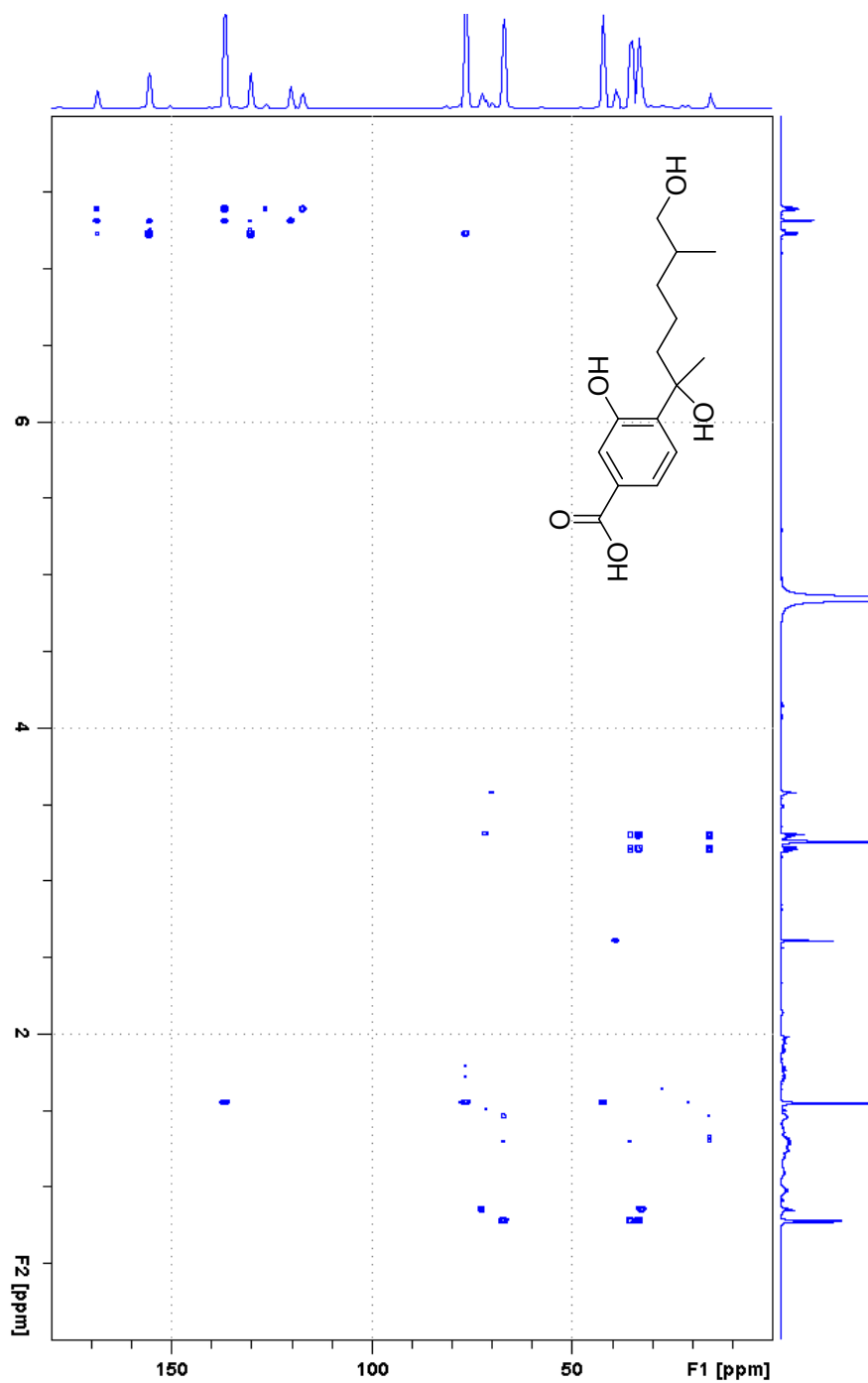


Figure S16. ^1H NMR spectrum of Cyclo(Trp-Phe) (**5**) in DMSO-d_6

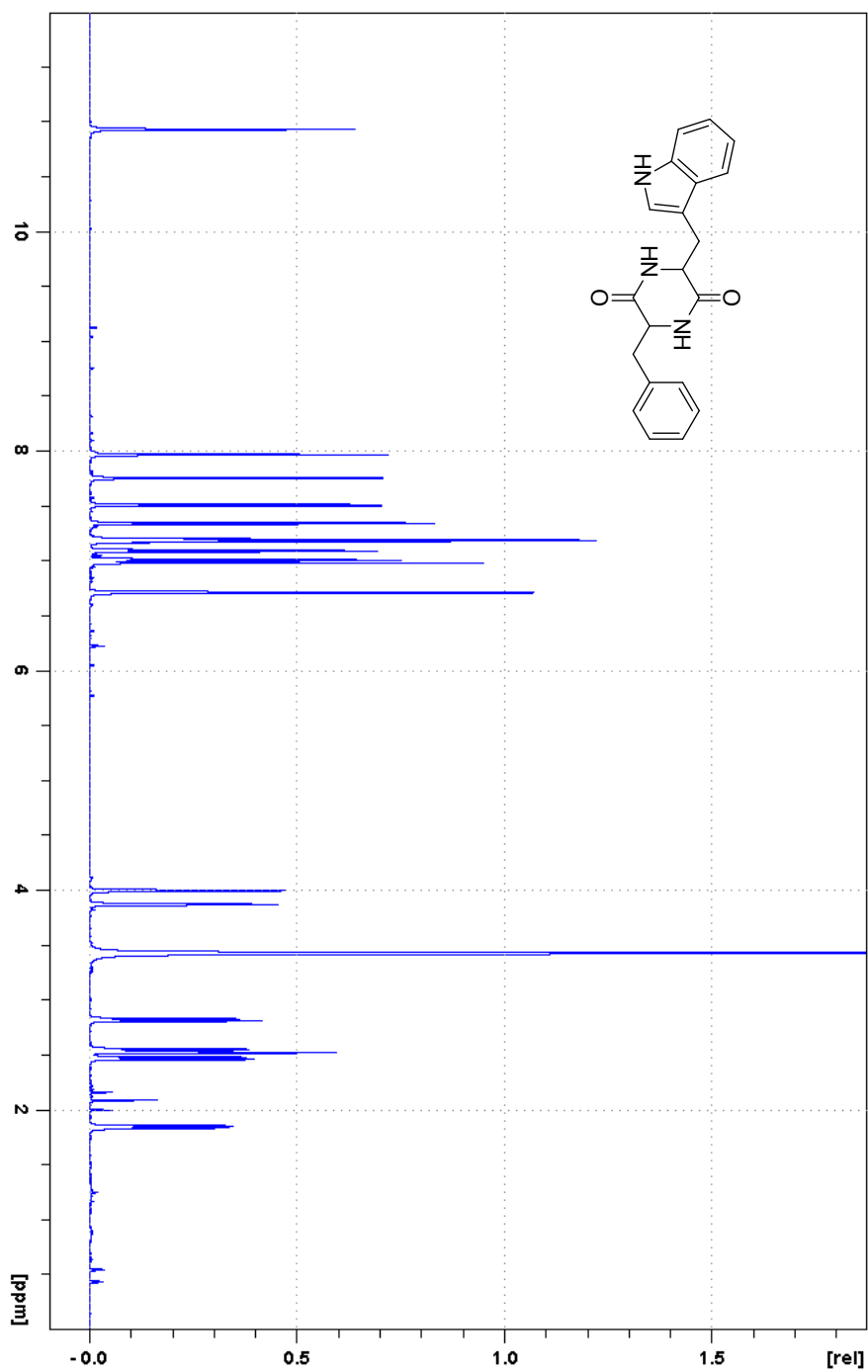


Figure S17. ^1H NMR spectrum of F12L0104_B3_B (**6**) in CDCl_3

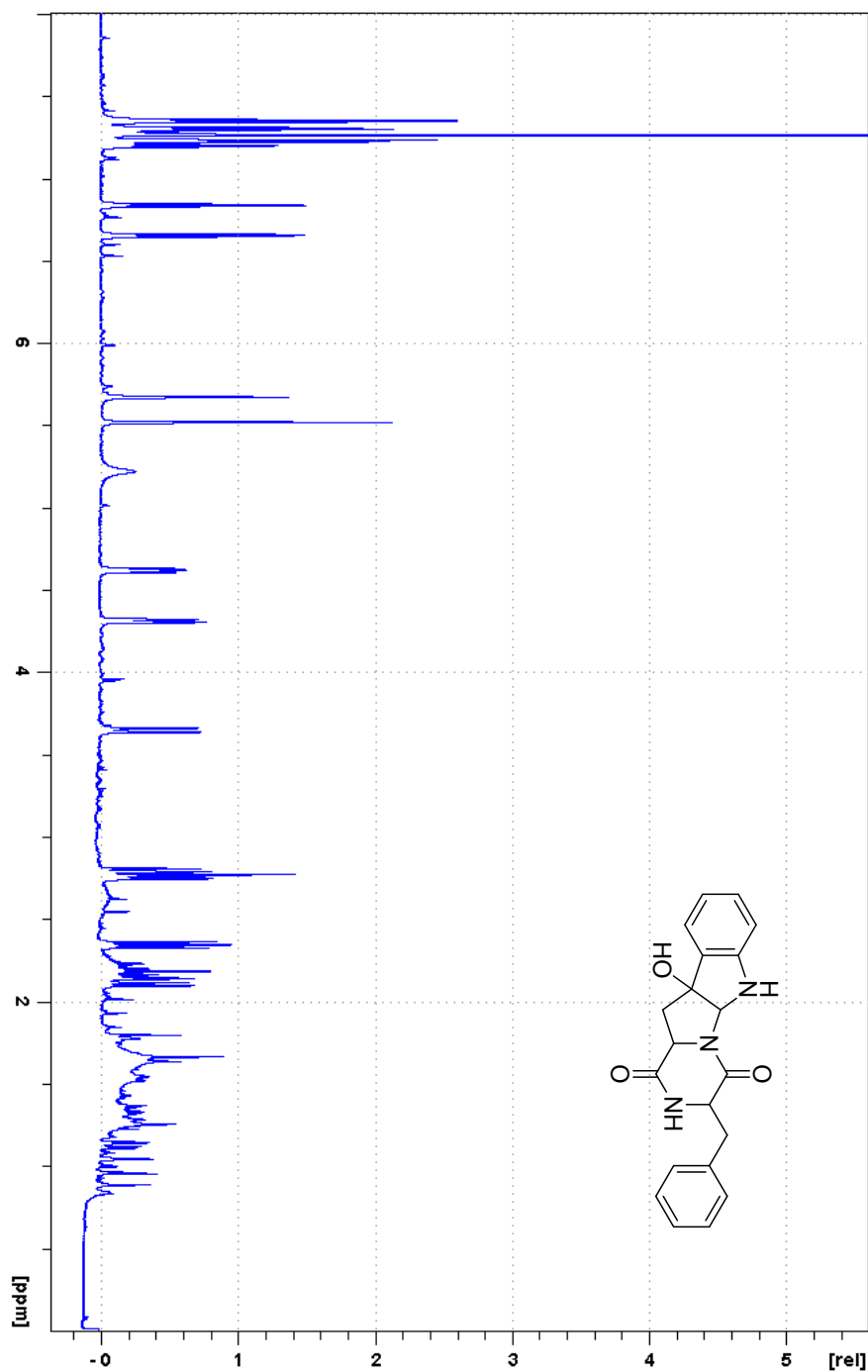


Figure S18. ^1H NMR spectrum of Demethylincisterol A₁ (**7**) in CDCl_3

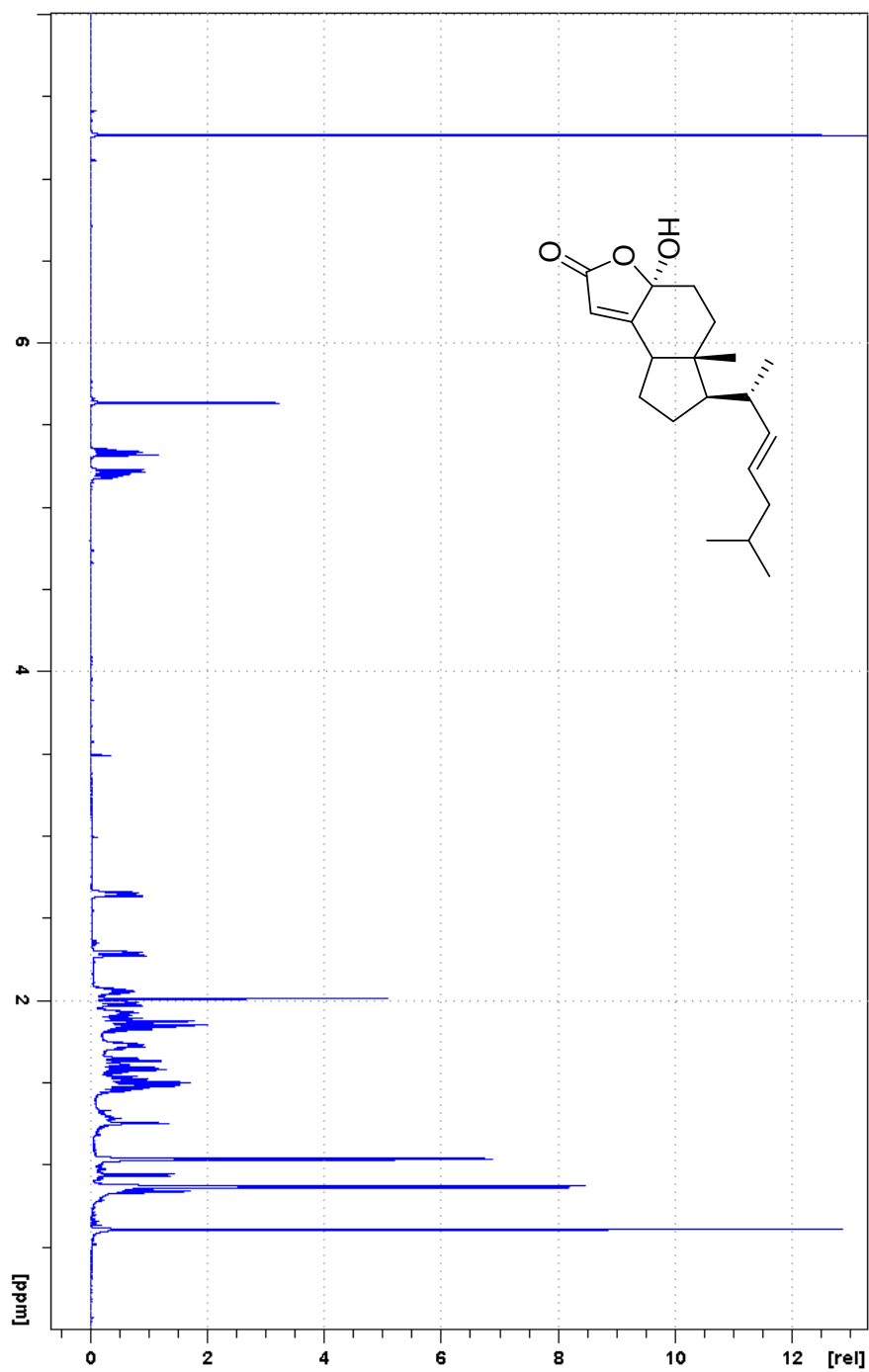


Figure S19. ^1H NMR spectrum of Demethylincisterol A₂ (**8**) in CD_3CD

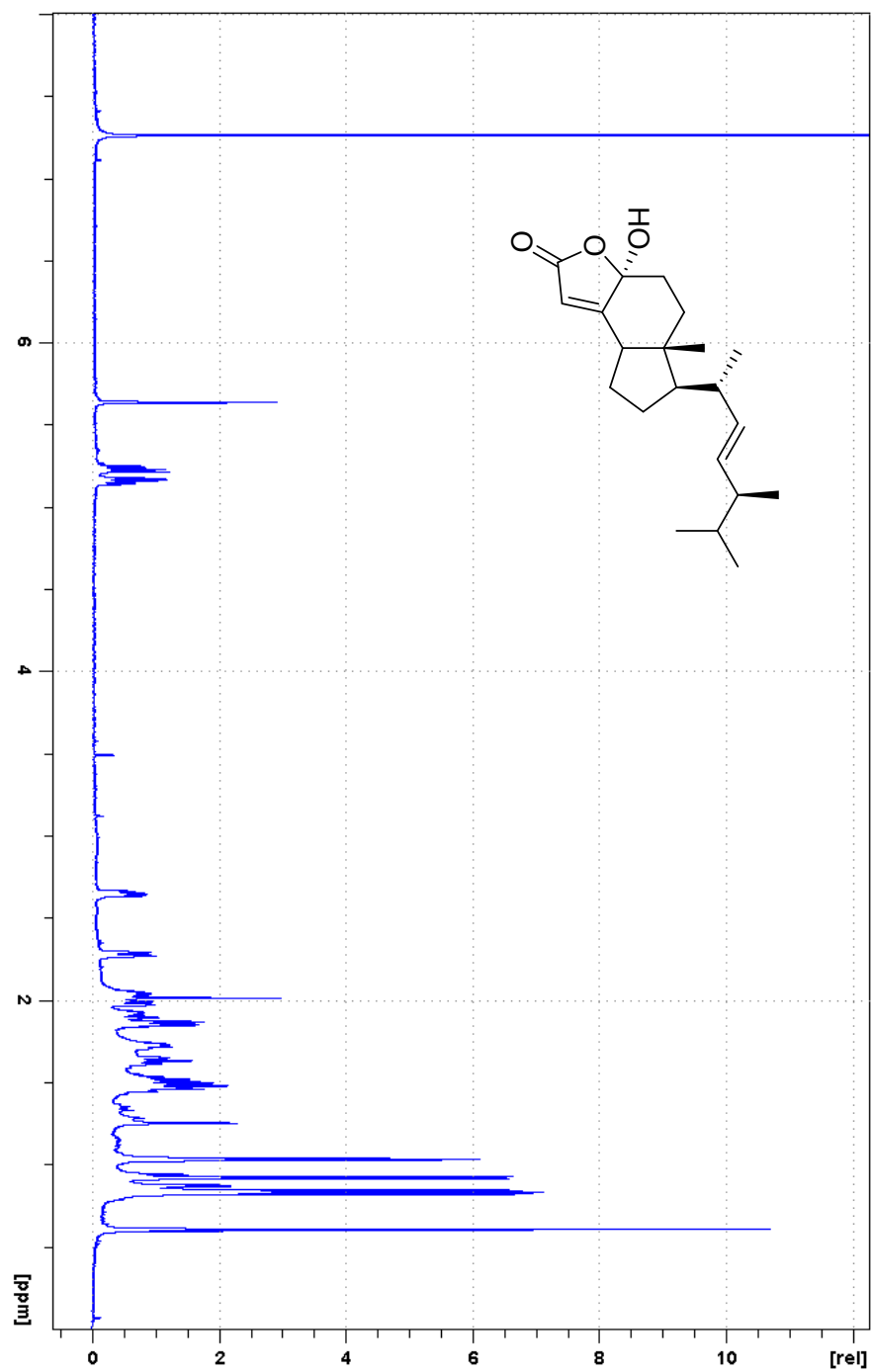
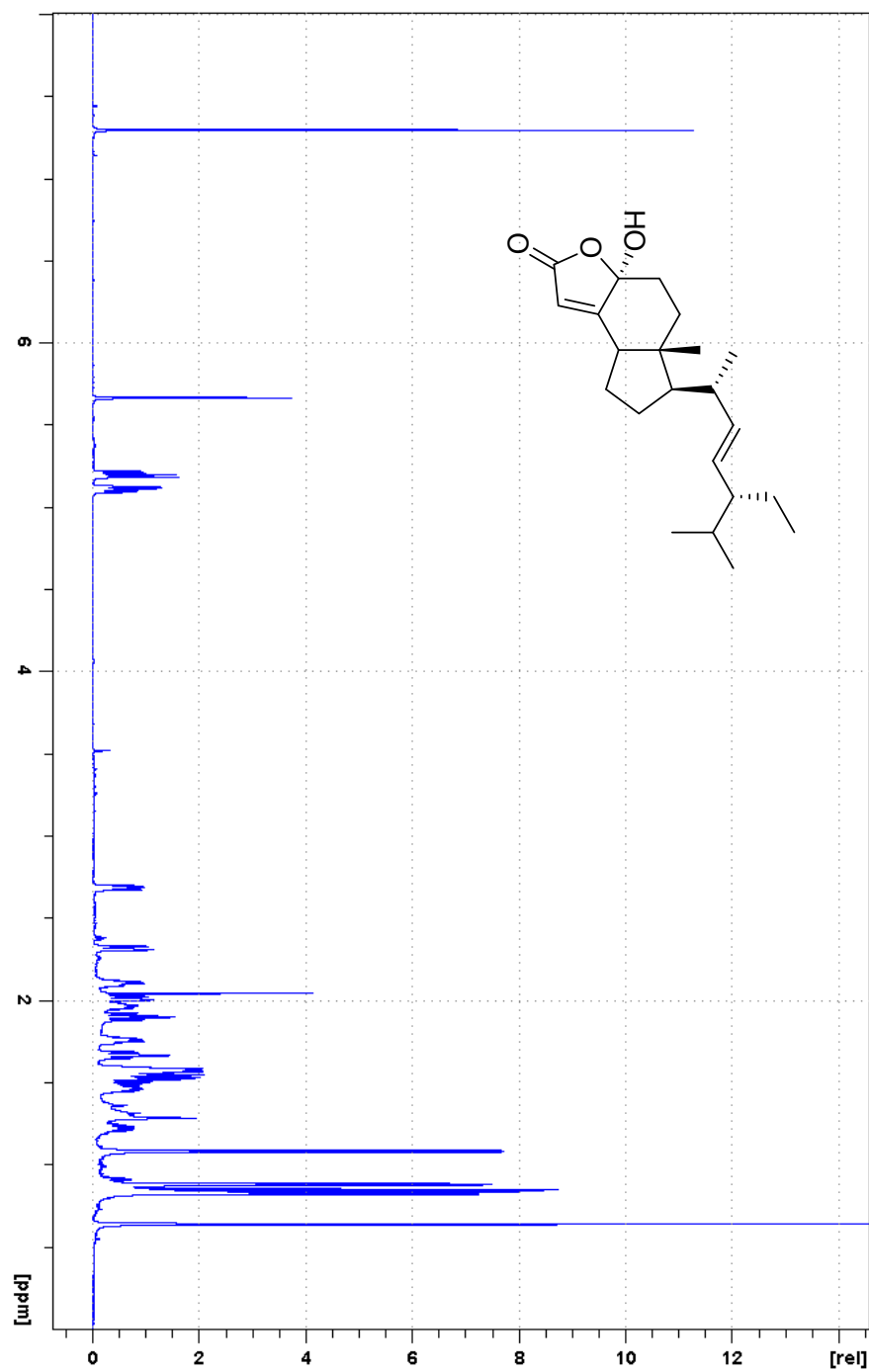


Figure S20. ^1H NMR spectrum of Demethylincisterol A₄ (**9**) in CD_3CD



한글초록

해양천연물은 해양생물이 생산하는 이차대사산물로 구조의 신규성이 높고 다양한 생리활성을 가지고 있어 신약개발의 중요한 자원으로 여겨져 왔다. 해양은 지구 표면적의 70 % 이상을 차지하고 있으며, 지구 전체 동물의 80 %에 달하는 다양한 해양생물들이 서식하는 것으로 알려져 있어 생물 원료의 보고라 할 수 있다. 그러나 바다라는 특수한 환경으로 인하여 해양생물 시료를 채집하는 것이 어려워 많은 연구가 진행되지 않았지만 최근 다양한 채집기술과 잠수정의 발전으로 해양 탐사가 용이해지면서 해양천연물 연구도 급속도로 발전하고 있다.

미국 캘리포니아에서 분리된 해양박테리아 *Nocardiopsis* sp.로부터 파이론 구조를 가지고 있는 신규한 마린노파이론 (Marinopyrone D,E)을 분리하였다. Marinopyrone D는 나이트릭 옥사이드 합성을 저해하여 면역활성 ($IC_{50}=13 \mu M$)을 보인다. 핵자기공명, 질량분석 등 다양한 분광학적 실험을 통하여 구조를 규명하였다.

해양 생물로부터 새로운 이차대사산물을 찾는 연구 중, 가거도에서 채집한 해면동물의 유래 곰팡이 *Aspergillus versicolor*로부터 프로스타글란딘 D_2 와 류코트리엔 C_4 의 합성을 저해하여 항염활성을 보이는 프랙션을 집중적으로 분리하여, 네 개의 알려진 세스퀴테페노이드와 펩타이드를 분리하였다. 화학구조는 핵자기공명, 질량분석, UV 분광법을 이용하여 결정하였다.

가거도에서 채집한 해면동물 *Homaxinella* sp.로부터 항균활성을 보이는 세 개의 알려진 스테롤 계열의 물질을 분리하였다.