

สารเคมีจากฟองน้ำของไทย *Mycale* sp.



นาย ฉัตรชัย วัฒนาภิรมย์สกุล

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรบริญาณศาสตร์มหาบัณฑิต
ภาควิชาเภสัชเวท
บัณฑิตวิทยาลัย จุฬาลงกรณ์มหาวิทยาลัย
พ.ศ. 2538
ISBN 974-631-382-7
ลิขสิทธิ์ของบัณฑิตวิทยาลัย จุฬาลงกรณ์มหาวิทยาลัย

CHEMICAL CONSTITUENTS FROM A THAI SPONGE, *MYCALE* SP.



Mr. Chatchai Watthanapiromsakul

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Sciences in Pharmacy
Department of Pharmacognosy
Graduate School
Chulalongkorn University
1995
ISBN 974-631-382-7

Thesis Title	Chemical Constituents from a Thai Sponge, <i>Mycale</i> sp.
By	Mr. Chatchai Watthanapiromsakul
Department	Pharmacognosy
Thesis Advisor	Mr. Khanit Suwanborirux, Ph.D.
Thesis Co-Advisor	Associate Professor Surattana Arnuoypol



Accepted by the Graduate School, Chulalongkorn University in partial fulfillment of the Requirements for the Master's Degree.

Santi Thoongsuwan Dean of Graduate School
(Associate Professor Santi Thoongsuwan, Ph.D.)

Thesis Committee

Chaiyo Chaichantipyuth Chairman
(Associate Professor Chaiyo Chaichantipyuth, M.Sc.)

Khanit Suwanborirux Thesis Advisor
(Mr. Khanit Suwanborirux, Ph.D.)

S. Amnuopol..... Thesis Co-Advisor
(Associate Professor Surattana Amnuopol, M.Sc.)

Rapepol Bavovada Member
(Associate Professor Rapepol Bavovada, Ph.D.)

พิมพ์ต้นฉบับบทด้วยอวิทยานิพนธ์ภายในกรอบสีเขียวนี้เพียงแผ่นเดียว

ฉัตรชัย รัตนาริมย์สกุล : สารเคมีจากฟองน้ำของไทย *Mycale* sp. (CHEMICAL CONSTITUTENTS FROM A THAI SPONGE, *MYCALE* sp.) อาจารย์ที่ปรึกษา : อาจารย์ ดร. คณิต สุวรรณริรักษ์, อาจารย์ที่ปรึกษาร่วม : รศ. สุรศนา อันวยผล, 183 หน้า.
ISBN 974-631-382-7

จากการแยกสารจากสิ่งสกัดในชั้นไคลอโรฟลูอีโนฟองน้ำไทย, *Mycale* sp. สามารถแยกสารใหม่ในกลุ่มเตียรอยด์คิโตนได้ 2 ชนิด ได้แก่ (24 R)-methylcholest-4-en-3-one-6 β -ol หรือ campest-4-en-3-one-6 β -ol และ (24 S)-ethylcholest-4-en-3-one-6 β -ol หรือ poriferast-4-en-3-one-6 β -ol และจากการแยกสารจากสิ่งสกัดในชั้นน้ำของฟองน้ำชนิดนี้ สามารถแยกสารในกลุ่มนิวคลีโอไซด์ได้ 4 ชนิด ได้แก่ thymine, uracil, thymidine, 2'-deoxyuridine ซึ่งสารทั้ง 4 ชนิดนี้ไม่เคยมีรายงานว่าพบในฟองน้ำสกุล *Mycale* มา ก่อน การพิสูจน์สูตรโครงสร้างของสารทั้ง 6 ชนิดนี้ทำได้โดยวิเคราะห์ข้อมูลจากスペkturm ของ uv, ir, ms, 1-D nmr และ 2-D nmr ร่วมกับการเปรียบเทียบข้อมูลกับสารอื่นที่มีโครงสร้างทางเคมีที่สัมพันธ์กันชนิดที่ทราบสูตรโครงสร้างแล้ว



ภาควิชา เกสชเวท
สาขาวิชา เกสชเวท
ปีการศึกษา 2537

ลายมือชื่อนิสิต ล้านารีบ วันที่ ๑๖๗๘
ลายมือชื่ออาจารย์ที่ปรึกษา
ลายมือชื่ออาจารย์ที่ปรึกษาร่วม

C575406 : MAJOR PHARMACOGNOSY

KEY WORD: *MYCALE*/ THAI SPONGE/ BIOACTIVITY/ CHEMICAL CONSTITUENTS

CHATCHAI WATTHANAPIROMSAKUL : CHEMICAL CONSTITUENTS FROM A THAI SPONGE, *MYCALE* sp. THESIS ADVISOR : Mr. KHANIT SUWANBORIRUX, Ph.D., THESIS CO-ADVISOR : ASSO. PROF. SURATTANA AMNUOYPOL, 183 pp. ISBN 974-631-382-7

Two new steroidal ketones, (24 R)-methylcholest-4-en-3-one-6 β -ol or campestan-4-en-3-one-6 β -ol and (24 S)-ethylcholest-4-en-3-one-6 β -ol or poriferast-4-en-3-one-6 β -ol were isolated from the dichloromethane extract of a Thai sponge, *Mycale* sp. and four known nucleosides, thymine, uracil, thymidine, and 2'-deoxyuridine were isolated from the aqueous extract of the same sponge. These nucleosides have not been previously found in the sponge genus, *Mycale*. The structure elucidations of the isolated compounds were achieved by analyses of the uv, ir, ms, 1-D nmr, and 2-D nmr spectral data, as well as comparison with other known related compounds.



ภาควิชา..... เภสัชเวท

ลายมือชื่อนิสิต..... คุณรัชนา ก้อนหินรัตน์สกุล

สาขาวิชา..... เภสัชเวท

ลายมือชื่ออาจารย์ที่ปรึกษา..... Khat Suwanbori ..

ปีการศึกษา..... 2537

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม..... S. Amnuoypol ..



ACKNOWLEDGEMENTS

I wish to express my grateful appreciation to my thesis advisor, Dr. Khanit Suwanborirux, Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, for his guidance, suggestion, encouragement, and kindness throughout the research studies.

I would like to acknowledge my sincere thanks to my thesis co-advisor, Associate Professor Surattana Amnuopol, Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University for her concern, kindly assistance and valuable advice.

I would like to thank Assistant Professor Noppamas Suppakun, Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University for her concern and valuable advice.

I would like to thank Professor Piamsak Menasaveta, the director of the Aquatic Resource Research Institute, Chulalongkorn University and Dr. Jun-ichi Tanaka, Department of Marine Sciences, University of the Ryukyus, for their support in the sample collection.

I would like to thank Dr. Amorn Petsom, Department of Chemistry, Chulalongkorn University for his kind support in MS experiment.

I would like to thank all NMR-operators of the Scientific and Technological Research Equipment Center, Chulalongkorn University for their helpful assistance.

I would like to thank the University Development Commission (UDC) for a scholarship throughout the two years of study, National Center for Genetic Engineering and Biotechnology, and the Graduate School of Chulalongkorn University for granting partial financial support .

I would also like to thank all my friends in the Department of Pharmacognosy for their contributions and hospitalities during the past three years.

Finally, I wish to express my infinite gratitude to my family for their love, understanding and encouragement.



CONTENTS

	Page
ABSTRACT (THAI).....	iv
ABSTRACT (ENGLISH).....	v
ACKNOWLEDGEMENTS.....	vi
CONTENTS.....	vii
LIST OF FIGURES.....	xi
LIST OF SCHEMES.....	xvi
LIST OF TABLES.....	xvii
ABBREVIATIONS.....	xviii
CHAPTER	
I INTRODUCTION.....	1
II HISTORICAL.....	4
Chemical Constituents of The Genus Mycale.....	4
- Nucleosides.....	4
- Terpenoids.....	4
- Amides.....	8
- Macrolides.....	8
- Halogenated acetogenins.....	10
- Fatty acids and Fatty aldehydes.....	11
- Miscellaneous compounds.....	12
Chemistry of Nucleosides.....	13
- Pyrimidine bases.....	13
- Purine bases.....	14
- Biosynthesis of pyrimidine nucleotides.....	14

- Biosynthesis of purine nucleotides.....	16
- Formation of deoxyribonucleotides.....	17
Distribution of Pyrimidine and Purine Nucleosides.....	17
- Sources of pyrimidine nucleosides and derivatives.....	17
- Chemical structure of pyrimidine nucleosides and derivatives.....	18
- Sources of purine nucleosides and derivatives.....	19
- Chemical structure of purine nucleosides and derivatives.....	20
Chemistry of Steroids.....	21
- Biosynthesis of steroids.....	24
- Conversion of cycloartenol.....	24
- Conversion of lanosterol.....	24
- Side chain formation.....	24
Distribution of Steroidal Ketones.....	25
III EXPERIMENTAL.....	26
Source of Sponges.....	26
General Techniques.....	26
- Thin-layer chromatography (TLC).....	26
- Column chromatography.....	26
- Spectroscopy.....	28
Bioactivity Determination.....	29
- Brine shrimp lethality activity.....	29
- Antimicrobial activity.....	30
- Cytotoxic activity.....	31

Extraction and Isolation.....	32
- Extraction.....	32
- Isolation of chemical constituents from the dichloromethane extract	33
- Isolation of chemical constituents from the aqueous extract... ..	35
Spectral Data of The Isolated Compounds.....	38
- Compound M-059.....	38
- Compound M-060.....	39
- Compound A-044.....	39
- Compound A-046.....	40
- Compound A-047.....	40
- Compound A-049.....	40
IV RESULTS AND DISCUSSION	42
Isolation of Chemical Constituents.....	42
- Isolation of chemical constituents from the dichloromethane extract.....	42
- Isolation of chemical constituents from the aqueous extract.....	44
Structure Elucidation of The Isolated Compound.....	46
- Structure elucidation of the isolated steroids.....	46
- Compound M-060.....	46
- Compound M-059.....	58
- Structure elucidation of the isolated nucleosides.....	66
- Compound A-044.....	66
- Compound A-046.....	68
- Compound A-047.....	70
- Compound A-049.....	75

V CONCLUSION.....	79
REFERENCES.....	80
APPENDIX.....	86
VITA.....	164

LIST OF FIGURES

Figure	Page
1 A Thai marine sponge <i>Mycale</i> sp.....	3
2 Pyrimidine bases.....	13
3 Purine bases.....	14
4 The <i>de novo</i> pathway of UTP and CTP formation.....	15
5 ^1H - ^1H correlation of M-060 observed in PDQF nmr spectrum..	50
6 The long range C-H correlation of compound M-060 observed from HMBC spectrum.....	51
7 Side chain C-H long range corralation of compound M-059 observed in HMBC spectrum.....	62
8 The long range C-H corralation of compound A-047 observed in HMBC spectrum.....	72
9 Result from NOE experiment of compound A-047.....	73
10 TLC chromatogram of fraction M-007 and M-055.....	87
11 HPLC chromatogram of fraction M-055.....	88
12 TLC chromatogram of fraction A-004, A-033, A-037 and compounds A-044, A-046, A-047, and A-049.....	89
13 The uv spectrum of compound M-060 (in chloroform).....	90
14 The ir spectrum of compound M-060 (film).....	91
15 The 500 MHz ^1H nmr spectrum of compound M-060 (in CDCl_3 , D_2O exchange).....	92
16 The 500 MHz ^1H nmr spectrum of compound M-060 (in CDCl_3 , D_2O exchange, expanded from 2.35 ppm- 2.60 ppm and 4.28-1.42 ppm).....	93
17 The 500 MHz ^1H nmr spectrum of compound M-060 (in CDCl_3 , D_2O exchange, expanded from 1.45 ppm-2.10 ppm).....	94
18 The 500 MHz ^1H nmr spectrum of compound M-060 (in CDCl_3 , D_2O exchange, expanded from 0.75 ppm-1.45 ppm).....	95
19 The 125 MHz ^{13}C nmr spectrum of compound M-060 (in CDCl_3).....	96

20	The 125 MHz ^{13}C nmr spectrum of compound M-060 (in CDCl_3 , expanded from 12 ppm-57 ppm).....	97
21	The 125 MHz DEPT 90 nmr spectrum of compound M-060 (in CDCl_3).....	98
22	The 125 MHz DEPT 135 nmr spectrum of compound M-060 (in CDCl_3 , expanded from 0 ppm- 58 ppm).....	99
23	The 500 MHz HSQC spectrum of compound M-060 (in CDCl_3).....	100
24	The 500 MHz HSQC spectrum of compound M-060 (in CDCl_3 , expanded from 10-60 ppm).....	101
25	The 500 MHz HSQC spectrum of compound M-060 (in CDCl_3 , expanded from 10-24 ppm).....	102
26	The 500 MHz ^1H , ^1H COSY (PDQF) spectrum of compound M-060, expanded from 4.28 ppm 4.38 ppm.....	103
27	The 500 MHz ^1H , ^1H COSY (PDQF) spectrum of compound M-060, expanded from 0.60 ppm 2.80 ppm.....	104
28	The 500 MHz ^1H , ^1H COSY (PDQF) spectrum of compound M-060, expanded from 0.70 ppm 2.10 ppm.....	105
29	The 500 MHz HMBC 4 Hz spectrum of compound M-060 (in CDCl_3), (1).....	106
30	The 500 MHz HMBC 4 Hz spectrum of compound M-060 (in CDCl_3), (2).....	107
31	The 500 MHz HMBC 4 Hz spectrum of compound M-060 (in CDCl_3), (3).....	108
32	The 500 MHz HMBC 4 Hz spectrum of compound M-060 (in CDCl_3), (4).....	109
33	The 500 MHz HMBC 4 Hz spectrum of compound M-060 (in CDCl_3), (5).....	110
34	The 500 MHz HMBC 4 Hz spectrum of compound M-060 (in CDCl_3), (6).....	111
35	The 500 MHz HMBC 4 Hz spectrum of compound M-060 (in CDCl_3), (7).....	112
36	The 500 MHz HMBC 4 Hz spectrum of compound M-060 (in CDCl_3), (8).....	113
37	The 500 MHz HMBC 4 Hz spectrum of compound M-060 (in CDCl_3), (9).....	114

38	The 500 MHz HMBC 4 Hz spectrum of compound M-060 (in CDCl ₃), (10).....	115
39	The eims spectrum of compound M-060.....	116
40	The uv spectrum of compound M-059 (in chloroform).....	117
41	The ir spectrum of compound M-059 (film).....	118
42	The 500 MHz ¹ H nmr spectrum of compound M-059 (in CDCl ₃).....	119
43	The 500 MHz ¹ H nmr spectrum of compound M-059 (in CDCl ₃ , expanded from 2.35 ppm-2.60 ppm and 4.32-4.38 ppm).....	120
44	The 500 MHz ¹ H nmr spectrum of compound M-059 (in CDCl ₃ , expanded from 1.45 ppm-2.10 ppm).....	121
45	The 500 MHz ¹ H nmr spectrum of compound M-059 (in CDCl ₃ , expanded from 0.75 ppm-1.40 ppm).....	122
46	The 125 MHz ¹³ C nmr spectrum of compound M-059 (in CDCl ₃).....	123
47	The 125 MHz ¹³ C nmr spectrum of compound M-059 (in CDCl ₃ , expanded from 12 ppm-56 ppm).....	124
48	The 500 MHz HSQC spectrum of compound M-059 (in CDCl ₃).....	125
49	The 500 MHz HSQC spectrum of compound M-059 (in CDCl ₃ , expanded from 10-60 ppm).....	126
50	The 500 MHz HSQC spectrum of compound M-059 (in CDCl ₃ , expanded from 10-26 ppm).....	127
51	The 500 MHz HMBC 6 Hz spectrum of compound M-059 (in CDCl ₃), (1).....	128
52	The 500 MHz HMBC 6 Hz spectrum of compound M-059 (in CDCl ₃), (2).....	129
53	The 500 MHz HMBC 6 Hz spectrum of compound M-059 (in CDCl ₃), (3).....	130
54	The 500 MHz HMBC 6 Hz spectrum of compound M-059 (in CDCl ₃), (4).....	131
55	The 500 MHz HMBC 6 Hz spectrum of compound M-059 (in CDCl ₃), (5).....	132
56	The 500 MHz HMBC 6 Hz spectrum of compound M-059 (in CDCl ₃), (6).....	133

57	The eims spectrum of compound M-059.....	134
58	The uv spectrum of compound A-044 (in methanol).....	135
59	The ir spectrum of compound A-044 (KBr disc).....	136
60	The 500 MHz ^1H nmr spectrum of compound A-044..... (in CD_3OD)	137
61	The 125 MHz ^{13}C nmr spectrum of compound A-044 (in CD_3OD).....	138
62	The eims spectrum of compound A-044.....	139
63	The uv spectrum of compound A-046 (in methanol).....	140
64	The ir spectrum of compound A-046 (KBr disc).....	141
65	The 500 MHz ^1H nmr spectrum of compound A-046 (in CD_3OD).....	142
66	The 125 MHz ^1H nmr spectrum of compound A-046 (in CD_3OD).....	143
67	The eims spectrum of compound A-046.....	144
68	The uv spectrum of compound A-047 (in methanol).....	145
69	The ir spectrum of compound A-047 (KBr disc).....	146
70	The 500 MHz ^1H nmr spectrum of compound A-047 (in CD_3OD).....	147
71	The 125 MHz ^{13}C nmr spectrum of compound A-047 (in CD_3OD).....	148
72	The 500 MHz HSQC spectrum of compound A-047.....	149
73	The 500 MHz HMBC 8 Hz spectrum of compound A-047 (in CD_3OD).....	150
74	The 500 MHz noe difference spectrum of compound A-047 [CD_3OD , irradiation at 7.81 ppm (H-6)].....	151
75	The 500 MHz noe difference spectrum of compound A-047 [CD_3OD , irradiation at 6.27 ppm (H-1')].....	152
76	The 500 MHz noe difference spectrum of compound A-047 [CD_3OD , irradiation at 4.39 ppm (H-3')].....	153
77	The 500 MHz noe difference spectrum of compound A-047 [CD_3OD , irradiation at 2.20 ppm ($\text{H}\beta$ -2')].....	154
78	The eims spectrum of compound A-047.....	155
79	The fab mass spectrum of compound A-047 (glycerol matrix).	156
80	The uv spectrum of compound A-049 (in methanol).....	157
81	The ir spectrum of compound A-049 (KBr disc).....	158

82	The 500 MHz ^1H nmr spectrum of compound A-049 (in CD_3OD).....	159
83	The 125 MHz ^{13}C nmr spectrum of compound A-049 (in CD_3OD).....	160
84	The 500 MHz HSQC spectrum of compound A-049.....	161
85	The eims spectrum of compound A-049.....	162
86	The fab mass spectrum of compound A-049 (glycerol matrix).163	

LIST OF SCHEMES

Scheme	Page
1 Isolation diagram of M-059 and M-060.....	43
2 Isolation diagram of A-044, A-046, A-047, and A-049.....	45
3 Proposed mass fragmentations of compound M-060.....	57
4 Proposed mass fragmentations of compound M-059.....	65
5 Proposed mass fragmentations of compound A-044.....	67
6 Proposed mass fragmentations of compound A-046.....	69
7 Proposed mass fragmentations of compound A-047.....	74
8 Proposed mass fragmentations of compound A-049.....	77

LIST OF TABLES

Table	Page
1 The Phospholipid fatty acids and fatty aldehydes.....	11
2 Sources of pyrimidine nucleosides and derivatives.....	17
3 Chemical structures of pyrimidine nucleosides and derivatives.....	18
4 Sources of purine nucleosides and derivatives.....	19
5 Chemical structures of purines nucleosides and derivatives.....	20
6 Sources of steroidal ketones.....	25
7 The carbon-proton correlations of M-060 observed in HSQC spectrum.....	48
8 Carbon and proton chemical shift assignment, proton proton correlations, and carbon proton long range correlations.....	52
9 Carbon chemical shifts of procesterol and M-060 (in CDCl ₃)... ..	54
10 Carbon chemical shifts of M-060, compound 2S and compound 2R (in CDCl ₃).....	55
11 Carbon chemical shifts of M-059 and M-060.....	59
12 Carbon chemical shifts of M-059, compound 1S, and compond 1R	60
13 The carbon-proton correlations of M-059.observed in the HSQC spectrum.....	60
14 Carbon and proton chemical shift assignment and carbon proton long range correlations.....	63
15 NOE enhancement for compound A-047.....	72
16 ¹ H assignments of compounds A-044, A-046, A-047, and A-049 (in CD ₃ OD).....	78
17 ¹³ C assignments of compounds A-044, A-046, A-047, and A-049 (in CD ₃ OD).....	78

ABBREVIATIONS



ϵ	= Molar absorptivity
ADP	= Adenosine 5' diphosphate
AMP	= Adenosine 5' monophosphate
ATP	= Adenosine 5' triphosphate
br. t	= Broad triplet (for nmr spectra)
°C	= Degree celsius
CDCl ₃	= Deuterated chloroform
CD ₃ OD	= Deuterated methanol
CH ₂ Cl ₂	= Dichloromethane
CHCl ₃	= Chloroform
¹³ C nmr	= Carbon-13 nuclear magnetic resonance
cm	= Centimeter
COSY	= Correlated spectroscopy
CTP	= Cytidine triphosphate
1-D	= One dimensional
2-D	= Two dimensional
d	= Doublet (for nmr spectra)
dd	= Doublets of doublet
ddd	= Doublets of doublets of doublet
dddd	= Doublets of doublets of doublets of doublet
DEPT	= Distortionless enhancement by polarization transfer
δ	= Chemical shift
dt	= Doublets of triplet
eims	= Electron impact mass spectrum
eV	= Electron volt
g	= Gram
¹ H-nmr	= Proton nuclear magnetic resonance
HPLC	= High performance liquid chromatography
HMBC	= ¹ H-detected heteronuclear multible bond coherence
HSQC	= ¹ H-detected high sensitive quantumn coherence
Hz	= Hertz
IC ₅₀	= 50 % Inhibition concentration

ir	= Infrared
<i>J</i>	= Coupling constant
kg	= Kilogram
l	= Liter
λ_{max}	= Wavelength at maxima absorption
M ⁺	= Molecular ion
MeOH	= Methanol
MHz	= Mega Hertz
μg	= Microgram
μm	= Micrometer
mg	= Milligram
mm	= Millimeter
MS	= Mass spectrum
NA	= Nutrient agar
ng	= Nanogram
nmr	= Nuclear magnetic resonance
No.	= Number
NOE	= Nuclear Over-Hausser effect
ν_{max}	= Wavenumber at maxima absorption
PDQF	= Phase sensitive double quantum frequency
pg	= Picogram
ppm	= Part per million
q	= Quatet (for nmr spectra)
s	= Singlet (for nmr spectra)
sp.	= Specy
spp.	= Species
SCUBA	= Self-contained underwater breathing apparatus
SDA	= Sabouraud dextrose agar
t	= Triplet (for nmr spectra)
TLC	= Thin layer chromatography
TSA	= Trypticase soy agar
UTP	= Uridine triphosphate
uv	= Ultraviolet