

CONTENTS

	Page
บทคัดย่อ	(3)
Abstract	(6)
Acknowledgement	(9)
The Relevance of the Research Work to Thailand	(10)
Contents	(11)
List of Tables	(13)
List of Illustrations	(16)
Abbreviations and Symbols	(21)
1 Introduction	1
1.1 Introduction	1
1.2 Review of literatures	1
1.2.1 Chemical constituents from the genera <i>Penicillium</i> and <i>Cordyceps</i>	1
1.2.2 Biological and pharmacological activities	2
1.2.3 Structures of compounds in Tables 1 and 2	8
1.3 The objectives	23
2 Experimental	25
2.1 Chemical and instruments	25
2.2 Extraction	25
2.3 Chemical investigation of <i>Penicillium</i> sp. BCC 7540	26
2.3.1 Purification of the broth extract	26
2.3.2 Purification of the mycelial extract	36
2.4 Chemical investigation of <i>Cordyceps militaris</i> BCC 2816	42
2.4.1 Purification of the broth extract	42
2.4.2 Purification of the mycelial extract	53
2.5 Chemical investigation of <i>Cordyceps militaris</i> BCC 2819	67
2.5.1 Purification of the broth extract	67
2.5.2 Purification of the mycelial extract	75
	(11)

CONTENTS (Continued)

	Page
3 Results and discussion	79
3.1 Structure determination of compounds isolated from <i>Penicillium</i> sp. BCC 7540	79
3.1.1 Compound VR-JOY2	79
3.1.2 Compound VR-JOY1	81
3.1.3 Compound VR-JOY3	82
3.1.4 Compound VR-JOY4	83
3.1.5 Compound VR-JOY5	85
3.1.6 Compound VR-JOY6	87
3.2 Structure determination of compounds isolated from <i>Cordyceps militaris</i> BCC 2816 and BCC 2819	89
3.2.1 Compound VR-JOY10	89
3.2.2 Compound VR-JOY9	92
3.2.3 Compound VR-JOY12	95
3.2.4 Compound VR-JOY13	96
3.2.5 Compound VR-JOY11	97
3.2.6 Compound VR-JOY7	99
3.2.7 Compound VR-JOY8	102
3.2.8 Compound VR-JOY14	105
3.2.5 Compound VR-JOY15	105
Reference	210
Vitae	215

LIST OF TABLES

Table	Page
1 Compounds isolated from <i>Penicillium</i> species and biological activity	2
2 Compounds isolated from <i>Cordyceps</i> species and biological activity	6
3 Fractions obtained from the broth extract of <i>Penicillium</i> species by column chromatography over Sephadex LH20	27
4 Subfractions obtained from A2 by column chromatography over silica gel	27
5 Subfractions obtained from A2-3 by column chromatography over reverse-phase C-18 silica gel	28
6 Subfractions obtained from A2-5 by column chromatography over silica gel	30
7 Subfractions obtained from A2-7 by column chromatography over silica gel	32
8 Subfractions obtained from A3 by column chromatography over silica gel	33
9 Subfractions obtained from A3-2 by column chromatography over silica gel	34
10 Subfractions obtained from A4 by column chromatography over silica gel	36
11 Fractions obtained from the mycelial extract by column chromatography over Sephadex LH20	37
12 Subfractions obtained from B2 by column chromatography over Sephadex LH20	37
13 Subfractions obtained from B2-2 by column chromatography over silica gel	38
14 Subfractions obtained from B2-3 by column chromatography on silica gel	39
15 Subfractions obtained from B3 by column chromatography on silica gel	40
16 Subfractions obtained from B4 by column chromatography on silica gel	41
17 Fractions obtained form the broth extract by column chromatography over Sephadex LH20	42
18 Subfractions obtained from C2 by column chromatography over silica gel	43
19 Subfractions obtained from C2-2 by column chromatography over silica gel	44
20 Subfractions obtained from C2-4 by column chromatography over silica gel	46
21 Subfractions obtained from C3 by column chromatography over silica gel	47
22 Subfractions obtained from C3-4 by column chromatography over silica gel	48

LIST OF TABLES (Continued)

Table	Page
23 Subfractions obtained from C4 by column chromatography over silica gel	50
24 Subfractions obtained from C4-2 by column chromatography over silica gel	51
25 Subfractions obtained from C4-4 by column chromatography over silica gel	52
26 Fractions obtained form the mycelial extract by column chromatography over Sephadex LH20	53
27 Subfractions obtained from D2 by column chromatography over silica gel	54
28 Subfractions obtained from D2-5 by column chromatography over Sephadex LH20	56
29 Subfractions obtained from D2-5-2 by column chromatography over silica gel	56
30 Subfractions obtained from D3 by column chromatography over silica gel	58
31 Subfractions obtained from D3-3 by column chromatography over silica gel	59
32 Subfractions obtained from D4 by column chromatography over silica gel	61
33 Subfractions obtained from D4-4 by column chromatography over silica gel	62
34 Subfractions obtained from D5 by column chromatography over silica gel	65
35 Subfractions obtained from chloroform soluble part of D6 by column chromatography over silica gel	66
36 Fractions obtained form the broth extract by column chromatography over Sephadex LH20	68
37 Subfractions obtained from E2 by column chromatography over silica gel	68
38 Subfractions obtained from E2-4 by column chromatography over silica gel	69
39 Subfractions obtained from E3 by column chromatography over silica gel	70
40 Subfractions obtained from E3-5 by column chromatography over silica gel	71
41 Subfractions obtained from E3-5-2 by column chromatography over silica gel	72
42 Subfractions obtained from E4 by column chromatography over silica gel	74
43 Fractions obtained form the mycelial extract by column chromatography over Sephadex LH20	75

LIST OF TABLES (Continued)

Table	Page
44 Subfractions obtained from F2 by column chromatography over silica gel	76
45 Subfractions obtained from F4 by column chromatography over silica gel	77
46 The NMR data of Compound VR-JOY2	81
47 The NMR data of Compound VR-JOY3 and <i>cis</i> -3,4-dihydro-4,8-dihydroxy-6,7-dimethoxy-3-methylisocoumarin	83
48 The NMR data of Compound VR-JOY4 and 2,3,4-trimethyl-5,7-dihydroxy-2,3-dihydrobenzofuran	85
49 The NMR data of Compound VR-JOY5 and terrein	86
50 The NMR data of Compound VR-JOY6	89
51 The NMR data of Compound VR-JOY10	91
52 The NMR data of VR-JOY9 and cephalosporolide C	94
53 The COSY and HMBC correlations of VR-JOY9	94
54 The NMR data of Compound VR-JOY12	96
55 The NMR data of Compound VR-JOY13	97
56 The NMR data of VR-JOY11	99
57 The NMR data of VR-JOY7 and cephalosporolide E	101
58 The HMBC correlations of VR-JOY7 and VR-JOY8	101
59 The NMR data of VR-JOY8 and cephalosporolide F	103
60 The NMR data of VR-JOY15	106

LIST OF ILLUSTRATIONS

Figure	Page
1 UV (MeOH) spectrum of VR-JOY2	107
2 FT-IR (neat) spectrum of VR-JOY2	107
3 ^1H NMR (500 MHz) (CDCl_3) spectrum of VR-JOY2	108
4 ^{13}C NMR (125 MHz) (CDCl_3) spectrum of VR-JOY2	109
5 NOEDIFF spectrum of VR-JOY2 after irradiation at δ_{H} 7.03	110
6 NOEDIFF spectrum of VR-JOY2 after irradiation at δ_{H} 6.64	111
7 2D HMQC (500 MHz) spectrum of VR-JOY2	112
8 HMBC (500 MHz) spectrum of VR-JOY2	113
9 ^1H NMR (300 MHz) (CDCl_3) spectrum of VR-JOY1	114
10 UV (MeOH) spectrum of VR-JOY3	115
11 FT-IR (KBr) spectrum of VR-JOY3	115
12 ^1H NMR (500 MHz) (CDCl_3) spectrum of VR-JOY3	116
13 ^{13}C NMR (125 MHz) (CDCl_3) spectrum of VR-JOY3	117
14 2D HMQC (300 MHz) spectrum of VR-JOY3	118
15 2D (300 MHz) HMBC spectrum of VR-JOY3	119
16 ^1H NMR (300 MHz) (CDCl_3) spectrum of VR-JOY4	120
17 ^{13}C NMR (75 MHz) (CDCl_3) spectrum of VR-JOY4	121
18 2D HMQC (300 MHz) spectrum of VR-JOY4	122
19 2D HMBC (300 MHz) spectrum of VR-JOY4	123
20 UV (MeOH) spectrum of VR-JOY5	124
21 FT-IR (neat) spectrum of VR-JOY5	124
22 ^1H NMR (300 MHz) (CDCl_3) spectrum of VR-JOY5	125
23 ^{13}C NMR (75 MHz) (Acetnone- d_6) spectrum of VR-JOY5	126
24 2D HMQC (300 MHz) spectrum of VR-JOY5	127
25 2D HMBC (300 MHz) spectrum of VR-JOY5	128
26 UV (MeOH) spectrum of VR-JOY6	129
27 FT-IR (KBr) spectrum of VR-JOY6	129
28 ^1H NMR (500 MHz) ($\text{CDCl}_3+\text{CD}_3\text{OD}$) spectrum of VR-JOY6	130

LIST OF ILLUSTRATIONS (Continued)

Figure	Page
29 ¹³ C NMR (125 MHz) (CDCl ₃ +CD ₃ OD) spectrum of VR-JOY6	131
30 NOEDIFF spectrum of VR-JOY6 after irradiation at δ _H 4.01	132
31 NOEDIFF spectrum of VR-JOY6 after irradiation at δ _H 2.43	133
32 2D HMQC (500 MHz) spectrum of VR-JOY6	134
33 2D HMBC (300 MHz) spectrum of VR-JOY6	135
34 Mass spectrum of VR-JOY6	136
35 UV (MeOH) spectrum of VR-JOY10	137
36 FT-IR (KBr) spectrum of VR-JOY10	137
37 ¹ H NMR (500 MHz) (CD ₃ OD) spectrum of VR-JOY10	138
38 COSY (300 MHz) spectrum of VR-JOY10	139
39 ¹³ C NMR (125 MHz) (CD ₃ OD) spectrum of VR-JOY10	140
40 DEPT spectrum of VR-JOY10	141
41 NOEDIFF spectrum of VR-JOY10 after irradiation at δ _H 5.95	142
42 NOEDIFF spectrum of VR-JOY10 after irradiation at δ _H 4.71	143
43 NOEDIFF spectrum of VR-JOY10 after irradiation at δ _H 4.52	144
44 2D HMQC (500 MHz) spectrum of VR-JOY10	145
45 2D HMBC (500 MHz) spectrum of VR-JOY10	146
46 Mass spectrum of VR-JOY10	147
47 UV (MeOH) spectrum of VR-JOY9	148
48 FT-IR (neat) spectrum of VR-JOY9	148
49 ¹ H NMR (300 MHz) (CDCl ₃ +CD ₃ OD) spectrum of VR-JOY9	149
50 COSY (300 MHz) spectrum of VR-JOY9	150
51 ¹³ C NMR (75 MHz) (CDCl ₃ +CD ₃ OD) spectrum of VR-JOY9	151
52 DEPT spectrum of VR-JOY9	152
53 NOEDIFF spectrum of VR-JOY9 after irradiation at δ _H 4.17	153
54 2D HMQC (300 MHz) spectrum of VR-JOY9	154
55 2D HMBC (300 MHz) spectrum of VR-JOY9	155
56 Mass spectrum of VR-JOY9	156

LIST OF ILLUSTRATIONS (Continued)

Figure	Page
57 FT-IR (neat) spectrum of VR-JOY12	157
58 ¹ H NMR (500 MHz) (CDCl ₃) spectrum of VR-JOY12	158
59 COSY (500 MHz) spectrum of VR-JOY12	159
60 ¹³ C NMR (125 MHz) (CDCl ₃) spectrum of VR-JOY12	160
61 DEPT spectrum of VR-JOY12	161
62 NOEDIFF spectrum of VR-JOY12 after irradiation at δ_{H} 4.11	162
63 NOEDIFF spectrum of VR-JOY12 after irradiation at δ_{H} 3.35	163
64 2D HMQC (500 MHz) spectrum of VR-JOY12	164
65 2D HMBC (500 MHz) spectrum of VR-JOY12	165
66 Mass spectrum of VR-JOY12	166
67 FT-IR (neat) spectrum of VR-JOY13	167
68 ¹ H NMR (500 MHz) (CDCl ₃) spectrum of VR-JOY13	168
69 COSY (300 MHz) spectrum of VR-JOY13	169
70 ¹³ C NMR (125 MHz) (CDCl ₃) spectrum of VR-JOY13	170
71 DEPT spectrum of VR-JOY13	171
72 NOEDIFF spectrum of VR-JOY13 after irradiation at δ_{H} 3.98	172
73 2D HMQC (500 MHz) spectrum of VR-JOY13	173
74 2D HMBC (500 MHz) spectrum of VR-JOY13	174
75 Mass spectrum of VR-JOY13	175
76 FT-IR (KBr) spectrum of VR-JOY11	176
77 ¹ H NMR (300 MHz) (CD ₃ OD) spectrum of VR-JOY11	177
78 COSY (300 MHz) spectrum of VR-JOY11	178
79 ¹³ C NMR (75 MHz) (CD ₃ OD) spectrum of VR-JOY11	179
80 2D HMQC (300 MHz) spectrum of VR-JOY11	180
81 2D HMBC (300 MHz) spectrum of VR-JOY11	181
82 Mass spectrum of VR-JOY11	182
83 X-ray structure of VR-JOY11	183
84 FT-IR (KBr) spectrum of VR-JOY7	184

LIST OF ILLUSTRATIONS (Continued)

Figure	Page
85 ¹ H NMR (300 MHz) (CD ₃ OD) spectrum of VR-JOY7	185
86 COSY (300 MHz) spectrum of VR-JOY7	186
87 ¹³ C NMR (75 MHz) (CD ₃ OD) spectrum of VR-JOY7	187
88 DEPT spectrum of VR-JOY7	188
89 NOEDIFF spectrum of VR-JOY7 after irradiation at δ_H 5.24	189
90 2D HMQC (300 MHz) spectrum of VR-JOY7	190
91 2D HMBC (300 MHz) spectrum of VR-JOY7	191
92 Mass spectrum of VR-JOY7	192
93 FT-IR (neat) spectrum of VR-JOY8	193
94 ¹ H NMR (300 MHz) (CDCl ₃ +CD ₃ OD) spectrum of VR-JOY8	194
95 COSY (300 MHz) spectrum of VR-JOY8	195
96 ¹³ C NMR (75 MHz) (CDCl ₃ +CD ₃ OD) spectrum of VR-JOY8	196
97 DEPT spectrum of VR-JOY8	197
98 2D HMQC (300 MHz) spectrum of VR-JOY8	198
99 2D HMBC (300 MHz) spectrum of VR-JOY8	199
100 Mass spectrum of VR-JOY8	200
101 UV (MeOH) spectrum of VR-JOY14	201
102 FT-IR (KBr) spectrum of VR-JOY14	201
103 ¹ H NMR (300 MHz) (CD ₃ OD) spectrum of VR-JOY14	202
104 ¹³ C NMR (75 MHz) (CD ₃ OD) spectrum of VR-JOY14	203
105 UV (MeOH) spectrum of VR-JOY15	204
106 FT-IR (neat) spectrum of VR-JOY15	204
107 ¹ H NMR (300 MHz) (CDCl ₃) spectrum of VR-JOY15	205
108 COSY (300 MHz) spectrum of VR-JOY15	206
109 ¹³ C NMR (75 MHz) (CDCl ₃) spectrum of VR-JOY15	207
110 2D HMQC (300 MHz) spectrum of VR-JOY15	208
111 2D HMBC (300 MHz) spectrum of VR-JOY15	209

LIST OF ILLUSTRATIONS (Continued)

Scheme	Page
1 Extraction procedure of a culture broth	26
2 Formation of cephalosporolide E (11) and cephalosporolide F (12) from cephalosporolide C (7)	104

ABBREVIATIONS AND SYMBOLS

<i>s</i>	=	<i>singlet</i>
<i>d</i>	=	<i>doublet</i>
<i>t</i>	=	<i>triplet</i>
<i>q</i>	=	<i>quartet</i>
<i>m</i>	=	<i>multiplet</i>
<i>brs</i>	=	<i>broad singlet</i>
<i>brd</i>	=	<i>broad doublet</i>
<i>dd</i>	=	<i>doublet of doublet</i>
<i>ddd</i>	=	<i>doublet of doublet of doublet</i>
<i>dt</i>	=	<i>doublet of triplet</i>
<i>td</i>	=	<i>triplet of doublet</i>
δ	=	chemical shift relative to TMS
<i>J</i>	=	coupling constant
$^{\circ}\text{C}$	=	degree celsius
R_f	=	retention factor
<i>g</i>	=	gram
<i>mg</i>	=	milligram
<i>mL</i>	=	milliliter
<i>nm</i>	=	nanometer
cm^{-1}	=	reciprocal centimeter (wave number)
<i>ppm</i>	=	part per million
λ_{max}	=	maximum wavelength
ν	=	absorption frequencies
<i>Hz</i>	=	Hertz
<i>MHz</i>	=	megaHertz
$[\alpha]$	=	specific rotation
H-n	=	position of protons
C-n	=	position of carbons
TLC	=	thin-layer chromatography

ABBREVIATIONS AND SYMBOLS (Continued)

UV	=	Ultraviolet
IR	=	Infrared
NMR	=	Nuclear Magnetic Resonance
1D NMR	=	one Dimensional Nuclear Magnetic Resonance
2D NMR	=	two Dimensional Nuclear Magnetic Resonance
MS	=	Mass spectroscopy
EIMS	=	electron impact mass spectroscopy
HRMS	=	high resolution mass spectroscopy
TOFMS	=	time of flight mass spectroscopy
HMQC	=	Heteronuclear Multiple Quantum Coherence
HMBC	=	Heteronuclear Multiple Bond Correlation
DEPT	=	Distortionless Enhancement by Polarization Transfer
NOE	=	Nuclear Overhauser Effect
NOEDIFF	=	Nuclear Overhauser Effect Difference Spectroscopy
TMS	=	tetramethylsilane
MeOH	=	methanol
NaOH	=	sodium hydroxide
CDCl ₃	=	deuteriochloroform
CD ₃ OD	=	tetradeuteromethanol
Acetone- <i>d</i> ₆	=	hexadeuteroacetone
CHCl ₃	=	chloroform
EtOAc	=	ethyl acetate
NaHCO ₃	=	sodium hydrogen carbonate
H ₂ O	=	water
ASA	=	anisaldehyde-sulfuric acid in acetic acid solution