CHAPTER III

EXPERIMENT

1. Chromatographic method

1.1 Analytical Thin-Layer Chromatography

Technique : one dimension, ascending

Adsorbent : silica gel F_{254} coated on aluminium sheet (E. Merck)

Layer thickness : 250 μm

Distance : 5 cm

Temperature : laboratory temperature (25-30 °C)

Detection : 1. Visual detection under daylight

2. Visual detection under ultraviolet light at wavelengths of 254 nm and 365 nm

1.2 Column chromatography

1.2.1 Flash column chromatography

Adsorbent : Silica gel 60 (No. 7734), particle size 0.063-0.200 nm (70-230 mesh ASTM) (E. Merck)

Packing method : Wet packing: The adsorbant was suspended in an eluant, poured into a column, and allowed to set tightly. pump before use.

Sample loading : The sample was dissolved in a small volume of the eluant and loaded on top of the column.

Detection : Fractions were examined by TLC technique in the same manner as described in section 1.1.

1.2.2 Gel filtration chromatography

Gel filter : Sephadex LH-20 (Pharmacia)

Packing method : Gel filter was suspended in the eluant and left standing to swell for 24 hours prior to use. It was then poured into the column and allowed to set tightly.

Sample loading : The sample was dissolved in a small volume of the eluant and applied on the top of the column.

Detection : Fractions were examined by TLC technique in the same manner as described in section 1.1.

2. Spectroscopy

2.1 Ultraviolet (UV) absorbtion spectroscopy

UV (in MeOH) spectra were obtained on a Shimadzu UV-160A spectrophotometer (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand).

2.2 Infrared (IR) absorption spectroscopy

IR spectra were recorded on a Perkin Elmer 2000 FT-IR spectrometer (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

2.3 Mass spectroscopy (MS)

The EIMS and FABMS spectra were obtained with a JEOL JMS – 700 mass spectrometer with direct inlet, operating at 10 kV inonization voltages (Meiji Pharmaceutical University, Japan).

2.4 Proton and Carbon Nuclear Magnetic Resonance (^{1}H and ^{13}C-NMR) Spectroscopy

^{1}H- NMR (300 MHz) and ^{13}C-NMR (75 MHz) spectra were obtained with a Bruker Avance DPX-300 FT-NMR spectrometer (Faculty of Pharmaceutical Sciences, Chulalongkorn University) and ^{1}H- NMR (500 MHz) and ^{13}C-NMR (125MHz) spectra were obtained with a Bruker Avance DPX-400 FT-NMR spectrometer (Meiji Pharmaceutical University, Japan).

The solvent for NMR spectra were deuterated chloroform ($CDCl_3$), or deuterated dimethyl sulfoxide (DMSO-d_6), or deuterated pyridine. Chemical shifts were reported in ppm scale using the chemical shift of the solvent as the reference signal.

3. Solvents

Throughout this work, all organic solvents were of commercial grade and redistilled prior to use.

Part A: The actinomycete *Micromonospora* sp. BTG10-2

1. Sample collection and isolation of actinomycetes

Soil samples were collected from peat swamp forests in the southern areas of Thailand, Trang Province, Phatthalung Province, Yala Province, and Narathiwat Province. The soil sample (0.5 g) was suspended in a test tube with 4.5 ml of sterile distilled water and heat in a water bath at 70 °C for 15 min. Then 0.5 ml of the suspension was transfered into 4.5 ml of sterile distilled water which was then diluted to a ten-fold dilution series. Of the final dilution step (10^{-3}), an aliquot of 100 µl was spread onto Starch-casein nitrate agar (SCA) plate supplemented with nystatin, novobiocin, and tetracycline (Brock *et al.*, 1993), and the plates were incubated at 30 °C for 7-21 days. The moist, pale yellow, orange, red brown, brown, blue green, purple, and black colonies of *Micromonospora* species were picked up and streaked for purification on yeast extract – malt extract agar plate (YMA) and were incubated at 30 °C for 7-21 days. The single colony was transferred to YMA slants and incubated at 30 °C for 14 days. The stock cultures were kept in cold room at 4 °C at Department of Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University.

2. Preparation of the crude extracts from microorganisms for antimicrobial primary screening

Twenty two microorganisms, selected from different sources of soils in Thailand, were tested for the antimicrobial activity by agar disc diffusion method against pathogenic bacteria and yeast.

The woking cultures of microorganisms were inoculated into a flask each containing 200 ml of yeast extract – malt extract (YM) broth, incubated on a rotary shaker at 200 rpm at room temperature for 7 days. The YM fermentation broths were filtered through a Buchner funnel packed with a layer of Kieselguhr. The filtrate was partitioned with ethyl acetate for three times, the ethyl acetate layer was concentrated under reduced pressure at 45°C to yield the crude ethyl acetate extracts for test antimicrobial activities.

3. Antimicrobial activity

Antimicrobial activity of the fractions and pure compounds were tested using the agar disc diffusion method. Activity was performed against pathogenic bacteria, *Micrococcus luteus* ATCC 9341, *Bacillus subtilis* ATCC 6633, *Pseudomonas aeruginosa* ATCC 27853, *Eschericia coli* ATCC 25922 and *Salmonella* sp., and yeast, *Candida albicans* ATCC 10231. All tested bacteria were cultivated on Muller – Hinton agar slants, MHA (Difco®), and the yeast was cultivated on Sabouraud dextrose agar slants, SDA (Difco®), at 37°C for 24 hours. All organic extracts prepared from microorganisms were dissolved in suitable solvents, at concentration 1 mg/disc and then applied on steriled paper discs. The dried paper discs were placed on the surface of the swabbed plates and incubated at 37°C for 24 hours. The diameters of inhibition zones were measured. Fractions exhibiting good antimicrobial activities were subsequently selected for further study.

4. Fermentation of *Micromonospora* sp. BTG 10-2

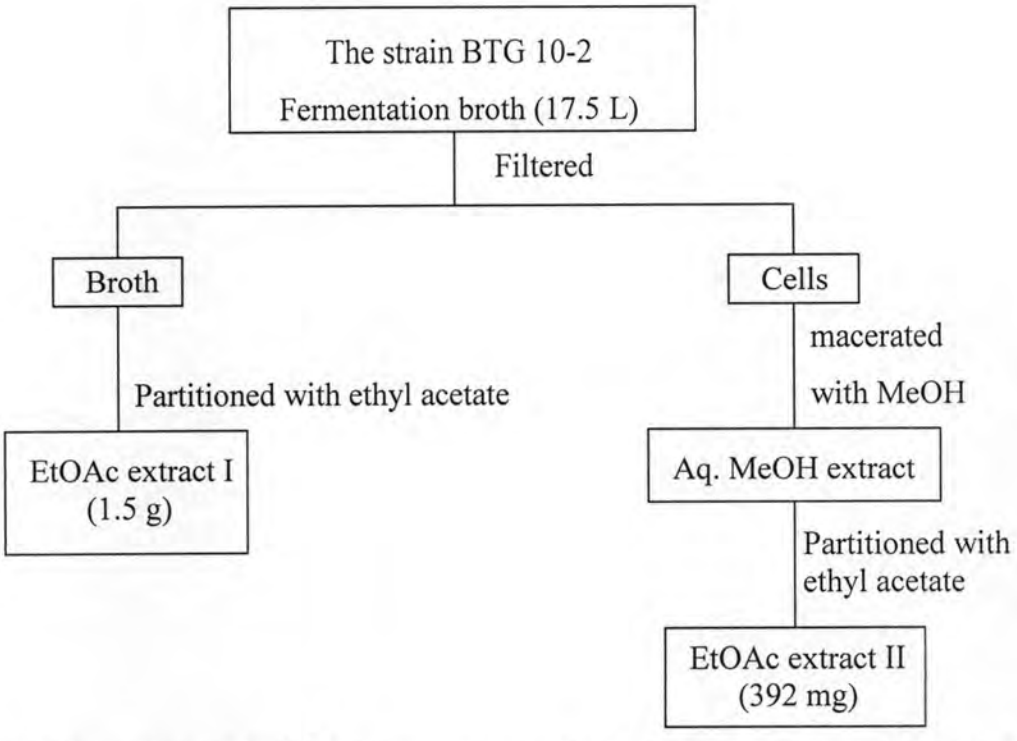
Three loopfuls of the *Micromonospora* sp. BTG 10-2 cultivated on yeast extract – malt extract (YM) slants were inoculated into a 200–ml of the seed medium (YM broth) in a 500–ml Erlenmeyer flask and shaken on a rotary shaker (200 rpm) at room temperature for 7 days. Two ml of the seed culture was inoculated into 200 ml of the

production medium (YM broth, added $CaCO_3$ 0.05 – 0.1% of the volume) in a 500–ml Erlenmeyer flask incubated on a rotary shaker (200 rpm) at room temperature for 12 – 14 days.

5. Extraction of the fermentation broth of *Micromonospora* sp. BTG 10-2

The YM fermentation broth (total, 17.5 L) was filtered through a Buchner funnel packed with a layer of Kieselguhr. The filtrate was partitioned with ethyl acetate for three times, and the ethyl acetate layer was concentrated under reduced pressure at 45°C to yield 1.5 g of the crude ethyl acetate extract I (yellow - brown oily liquid).

The cells were macerated with methanol. Then the filtered methanol part was concentrated under reduced pressure to give an aqueous methanol. The aqueous methanol was partitioned with ethyl acetate for three times, and the ethyl acetate layer was concentrated under reduced pressure at 45°C to yield 392 mg of the crude ethyl acetate extract II (orange - brown oily liquid). (Scheme 3.1)



Scheme 3.1 Extraction of the YM fermentation broth of *Micromonaspra* sp. BTG 10-2

All extracts were examined for antimicrobial activity by the method as described in section 6. The EtOAc extract I of BTG 10-2 from the fermentation broth exhibited antibacterial activity against gram positive bacteria including *Micrococcus*

luteus ATCC 9341 and *Bacillus subtilis* ATCC 6633 with clear zones of 35 and 29 mm and against gram negative bacteria, *Pseudomonas aeruginosa* ATCC 27853, *Eschericia coli* ATCC 25922 and *Salmonella* sp. with clear zones of 16, 9 and 8 mm at concentration 1 mg/disc (disc diffusion method), respectively. The EtOAc extract was subsequently purified by chromatographic techniques.

6. Fractionation of the ethyl acetate extract

The EtOAc extract I was isolated on a silica gel quick column (Ø = 5 cm.), using dry packing and dry loading method. Gradient elution with CH_2Cl_2 and MeOH mixtures was used. Fractions (20 ml each) were collected and examined. Fraction combination was guided by TLC on silica gel plates with CH_2Cl_2 and MeOH (9:1) as a developing solvent. Fractions with the same TLC pattern were pooled and dried. Seven fractions (M1-7) were obtained (Table 3.1).

Table 3.1 Fractionation of the EtOAc extract of *Micromonaspra* sp. BTG 10-2 by a silica gel quick column chromatography

Fraction code	Number of fractions	Percentage of MeOH in CH_2Cl_2	Volume (ml) of CH_2Cl_2:MeOH	Weight (mg)
M1	1-10	0	200	521.6
	11-13	5	60	
M2	14-17	5	80	447.2
M3	18-22	5	100	79.7
M4	23-68	5	900	75.5
M5	69-86	5	340	12.4
	87-91	7	80	
M6	92-98	10	140	34.7
	99-106	20	160	
M7	107-108	20	40	55.7
	109-114	50	120	
	115-122	100	160	

Fractions M2, M3, M4, M5, M6 and M7 showed antibacterial activity against *Micrococcus luteus* ATCC 9341 with 8, 20, 30, 30, 25 and 16 mm of inhibition zones, and showed antibacterial activity against *Bacillus subtilis* ATCC 6633 with clear zones of 7, 14, 19, 19, 14 and 7 mm, respectively at the concentration of 1 mg/disc. Therefore fraction M4 (75.5 mg) was further purified on a Sephadex LH-20 column (1.5 x 50 cm). A mixture of CH_2Cl_2 and MeOH, ratio 9:1, was used as an eluant. Fractions of 20 ml were collected. They were then combined according to their TLC pattern (silica gel plate with CH_2Cl_2 and MeOH (9:1) as the developing solvent) to give three fractions (M41-M43) (Table 3.2).

Table 3.2 Fractionation of M4 by a Sephadex LH-20 column

Fraction code	Number of fraction	Volume (ml) CH_2Cl_2:MeOH	Weight (mg)
M41	1-2	40	41.9
M42	3-5	60	23.5
M43	6-9	80	8.3

Fraction M41 was further purified on a Sephadex LH-20 column (1.5 x 50 cm). MeOH was used to elute the column. Fractions of 20 ml were collected. Fraction combination was guided by TLC (Silica gel) pattern with 10% MeOH in CH_2Cl_2 as the developing solvent. Five fractions (M411-415) were obtained (Table 3.3).

Table 3.3 Fractionation of M41 by a Sephadex LH-20 column

Fraction code	Number of fraction	Volume of MeOH (ml)	Weight (mg)
M411	1-2	40	4.1
M412	3	20	11.6
M413	4	20	20.2
M414	5	20	4.0
M415	6-7	40	1.1

Fraction M413 showed antibiotic activity against *Bacillus subtilis* ATCC 6633 with inhibition zones of 18 mm at the concentration of 1 mg/disc. So, fraction M413 was further purified on a Sephadex LH-20 column (1.5 x 50 cm). MeOH was used to elute the column. Fractions of 1 ml were collected. After the combination of the fractions with the similar TLC pattern, seven fractions (M4131-4137) were obtained (Table 3.4).

Table 3.4 Fractionation of M413 by a Sephadex LH-20 column

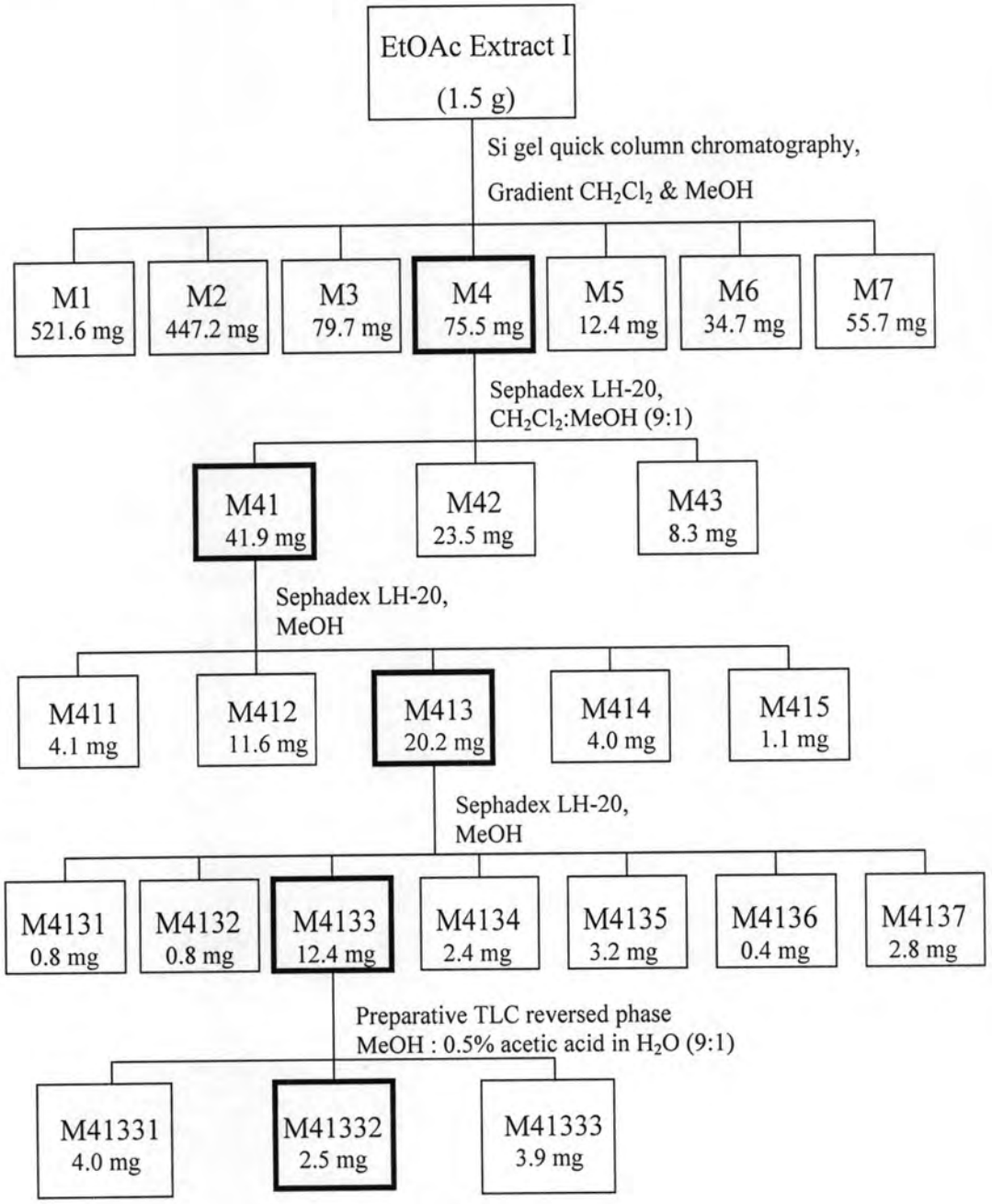
Fraction code	Number of fraction	Volume of MeOH (ml)	Weight (mg)
M4131	1-3	3	0.8
M4132	4	1	0.8
M4133	5-8	4	12.4
M4134	9-13	5	2.4
M4135	14-16	3	3.2
M4136	17-18	2	0.4
M4137	19-22	4	2.8

Fraction M4133 was purified by the preparative TLC reverse phase (10 x 20 cm) with a mixture of MeOH and 0.5% acetic acid in water (9:1) as the solvent system. There was a large yellow band appearing on the TLC plate after the second development, and this yellow band was further separated into three bands (M41331-41333). The compounds were eluted from the TLC plate by MeOH (Table 3.5).

Table 3.5 Fractionation of M4133 by the preparative TLC reversed phase

Fraction code	Bands	Weight (mg)
M41331	Up	4.0
M41332	Middle	2.5
M41333	Down	3.9

All fractions obtained from *Micromonospora* sp. BTG 10-2 was kept in the desiccator for further investigation.



Scheme 3.2 Fractionation of the EtOAc extract of *Micromonospora* sp. BTG 10-2

Part B: The endophytic fungus *Exserohilum rostratum* RNAS5

1. Determination of antimicrobial activities

1.1. Determination of antibacterial activities by broth microdilution method

Broth microdilution method was used to determine antibacterial activities of rostratin A, W3, and R4/2-7, as described in NCCLS M7-A4. Test bacteria used in this study were *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. Each bacterial strain was grown in tryptic soy broth at 37 °C for 2-3 hours and adjusted to match the turbidity of 0.5 McFarland turbidity standard (OD_{625} 0.1), resulting in a suspension containing approximately 10^8 CFU/ml. The adjusted bacterial suspension was further diluted in Mueller-Hinton broth to obtain 10^6 CFU/ml. A 50-µl inoculum was dispersed into each well that contained 50 µl of test compound.

The compounds were tested at the final concentration of 64 µg/ml. The experiment was done in duplicate. After incubation at 37 °C for 24 hours, a 20-µl of *p*-iodonitrotetrazolium (INT) solution (1 mg/ml) was added into each well. The assay plates were further incubated for 1 hour. Violet color developed in each well indicated the growth of tested organism. The well that showed no change in color indicated antibacterial activity of the test compound.

1.2. Determination of antifungal activities by agar disc diffusion method

Antimicrobial activity of the fractions and the pure compounds were tested using the agar disc diffusion method. Activity was performed against yeast, *Candida albicans* ATCC 10231. The yeast was cultivated on Saborouraud dextrose agar slant, SDA (Difco®), at 37°C for 24 hours. All organic extracts prepared from microorganisms were dissolved in suitable solvents and then applied on steriled paper discs at concentration 1 mg/disc. The dried paper discs were placed on the surface of the swabbed plates and incubated at 37°C for 24 hours. The diameter of each inhibition zones was measured. Fractions exhibiting good antimicrobial activities were subsequently selected for further study.

1.3. Determination of antifungal activities by broth microdilution method

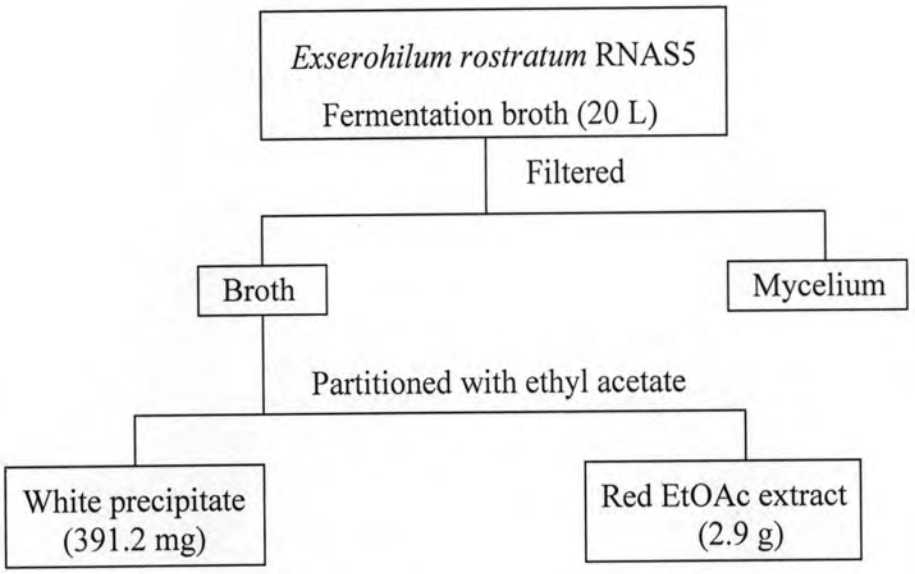
Anti- *C. albicans* activity of R5-9, W3, and R4/2-7, was determined by broth microdilution method as described in NCCLS M27-A2 (2). *Candida albicans* ATCC 10231 was grown on Sabouraud's dextrose agar at 30 °C for 24 hours. Five single isolated colonies (~1-mm. diameter) were suspended in sterile normal saline solution and adjusted to match the turbidity of 0.5 McFarland turbidity standard (OD_{625} 0.1), resulting in a suspension containing approximately $1x10^6$ - $5x10^6$ CFU/ml. The adjusted yeast suspension was further diluted in RPMI1640 medium to yield $1x10^3$ - $5x10^3$ CFU/ml. A 100-µl inoculum was dispensed into each well that contained 100 µl of test compound.

The compounds were tested at the final concentration of 64 µg/ml. The experiment was done in duplicate. After incubation at 37 °C for 24 hours, a 20-µl of *p*-iodonitrotetrazolium (INT) solution (1mg/ml) was added into each well. The assay plates were further incubated for 24 hours. Violet color developed in each well indicated the growth of test organism. The well that showed no change in color indicated anti- *C. albicans* activity of the test compound.

2. Extraction of the fermentation broth of *Exserohilum rostratum* RNAS5

The endophytic fungus *Exserohilum rostratum* RNAS5 was grown in Sabouraud's dextrose broth (SDB) in the 1-L Erlenmeyer flask under stationary condition at 25 °C for 21 days. The culture broth (total, 20 liters) was separated from mycelium by filtering through 4 layers of cotton gauze. The broth filtrate was partitioned with ethyl acetate for three times, the ethyl acetate layer was concentrated under reduced pressure at 45 °C to yield 3.9 g of the crude ethyl acetate extract as a reddish brown oil and 391.2 mg of white precipitates. (Scheme 3.3)

All extracts were examined for antimicrobial activity using the method as described in Section 1.



Scheme 3.3 Extraction of the fermentation broth of *Exserohilum rostratum* RNAS5

3. Fractionation of the EtOAc extract from *Exserohilum rostratum* RNAS5

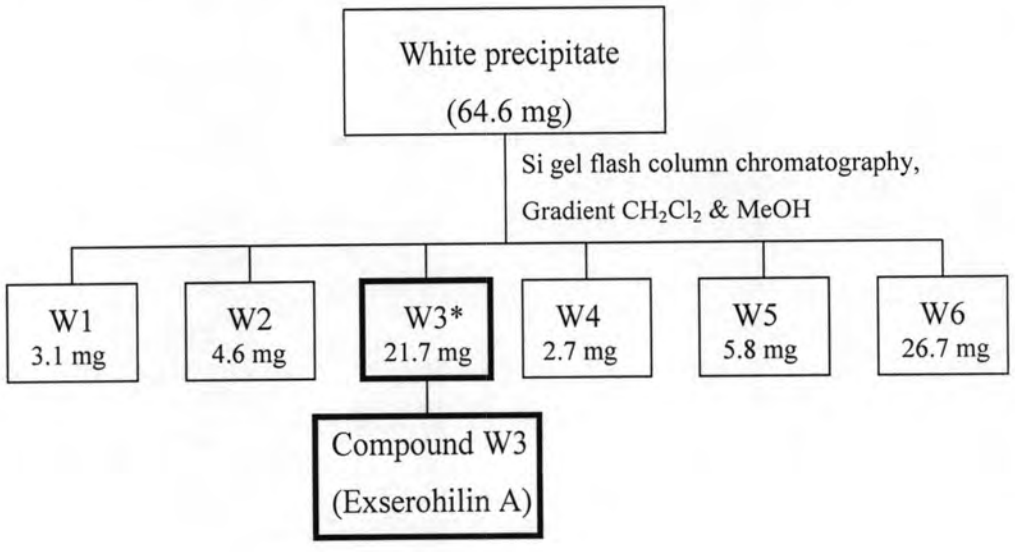
3.1 Isolation of compound W3 from *Exserohilum rostratum* RNAS5

The crude white precipitate from the fermentation broth was slightly dissolved in organic solvents. In this case, we used a large volume of a mixture of CH_2Cl_2, MeOH and EtOAc. Afterward, the solution was triturated with kieselguhr and dried. The powder was divided into eleven parts, and each was loaded on a Silica gel column (2.5 x 16 cm), using wet packing and dry loading method. Gradient elution with CH_2Cl_2 and MeOH mixtures was used. Fractions (25 ml each) were collected. Fraction combination was guided by TLC on a silica gel plate with CH_2Cl_2 and MeOH (15:1) as the developing solvent. Fractions with similar TLC patterns were pooled and dried. Six fractions (W1-6) were obtained (scheme 3.4 & Table 3.6).

Table 3.6 Isolation of compound **W3** by a silica gel column

Fraction code	Number of fractions	Percentage of MeOH in CH_2Cl_2	Volume (ml) of CH_2Cl_2:MeOH	Weight (mg)
W1	1-3	0	75	3.1
W2	4-6	3	75	4.6
W3	7-9	3.23	75	21.7
	10-11	5	50	
W4	12-18	10	175	2.7
W5	19-20	30	50	5.8
	21-22	50	50	
W6	23-26	100	100	26.7

Fraction W3 gave a white precipitate (21.7 mg). Biological activities and structure elucidation of this compound were described in Chapter IV.



*white precipitate

Scheme 3.4 Isolation of compound **W3 (exserohilin A)** from *Exserohilum rostratum* RNAS5

3.2 Isolation of compounds R4/2-7 and R5-9 from *Exserohilum rostratum* RNAS5

The red EtOAc extract (2.9 g) was separated on a silica gel column (Ø = 5 cm.), using dry packing and dry loading method. Gradient elution with EtOAc, Hexane, CH_2Cl_2 and MeOH mixtures was used. Fractions (25 ml each) were collected, and examined by TLC on a silica gel plate with CH_2Cl_2 and MeOH (15:1) as a developing solvent. Fractions with similar TLC patterns were pooled and dried. Eight fractions (R1-8) were obtained (Table 3.7).

Fraction R4 contained a white precipitate (R4) and a brown orange oily liquid (R4/2) while fraction R6 contained a yellow precipitate (R6) and a brown oily liquid (R6/2).

Table 3.7 Isolation of compounds **R4/2-7** and **R5-9** from *Exserohilum rostratum* RNAS5 by a silica gel column

Fraction code	Number of fractions	Ratio of EtOAc:Hexane:MeOH:CH_2Cl_2	Volume (ml) of eluant	Weight (mg)
R1	1-9	9:1:0:0	225	36.1
R2	10-13	9:1:0:0	100	854
R3	14-17	9:1:0:0	100	471.8
R4 (R4/2)	18-35	9:1:0.5:0	450	104.8
				701.5
R5	36-41	9:1:0.5:0	125	212.7
R6 (R6/2)	42-48	9:1:0.5:0	175	11.7
	49-54	0:0:1:9	150	259.6
R7	55-61	0:0:1:9	175	283.7
	62-65	0:0:3:7	100	
R8	66-70	0:0:3:7	125	181.1
	71-79	0:0:1:1	225	
	80-84	0:0:1:0	125	

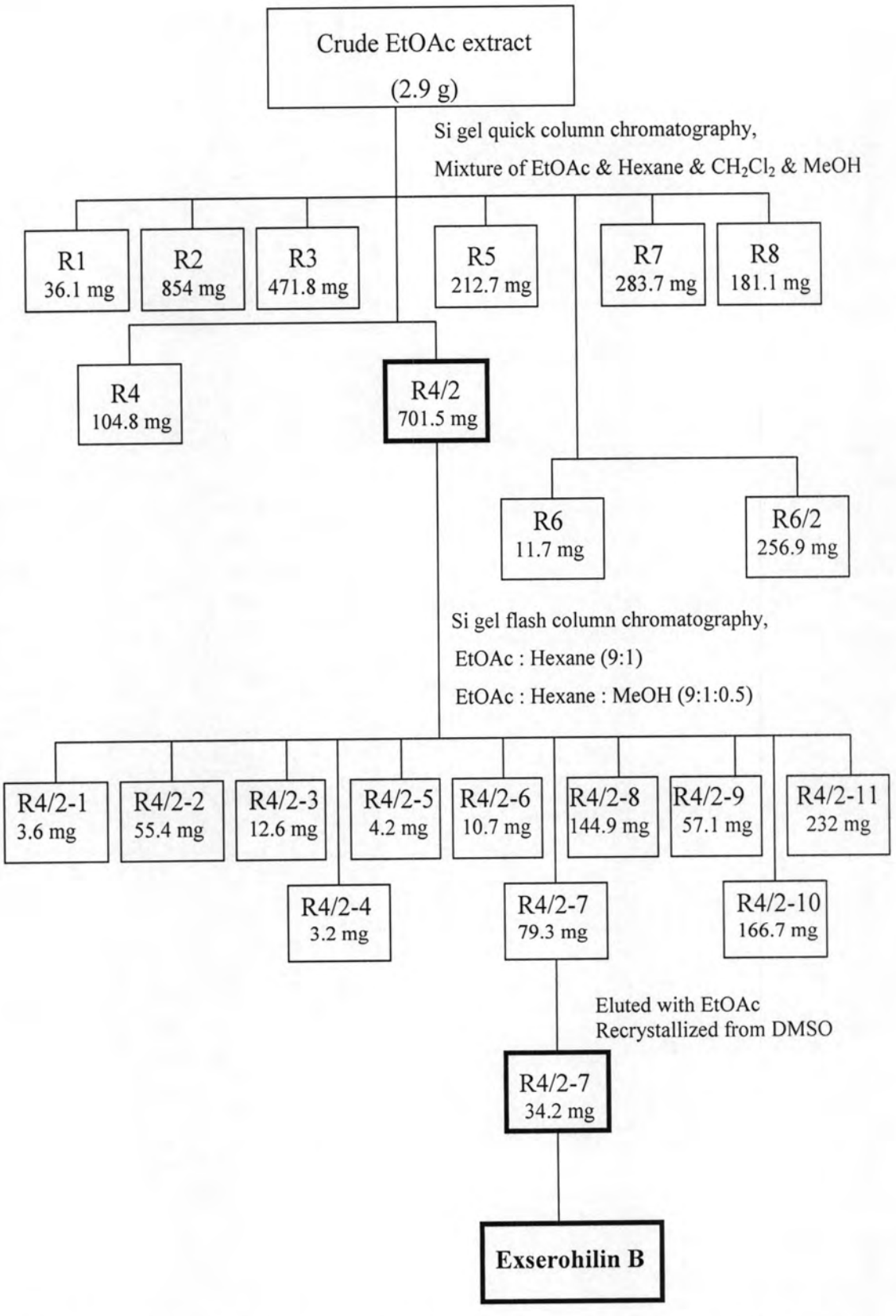
Fraction R4/2 was further purified on a silica gel flash column (2.5 x 16 cm), using EtOAc-Hexane (9:1), EtOAc-Hexane-MeOH (9:1:0.5), and MeOH as the eluting solvents, respectively. Fractions (25 ml each) were collected, and examined. Fraction combination was guided by a TLC on Silica gel plate with EtOAc and Hexane (9:1) as the developing solvent. Fractions with the similar TLC patterns were pooled and dried. Eleven fractions (R4/2-1-11) were obtained (Table 3.8).

Table 3.8 Isolation of compound **R4/2-7** from *Exserohilum rostratum* RNAS5 by a silica gel flash column

Fraction code	Number of fractions	Ratio of EtOAc:Hexane:MeOH	Volume (ml) of eluent	Weight (mg)
R4/2-1	1-2	9:1:0	50	3.6
R4/2-2	3	9:1:0	25	55.4
R4/2-3	4	9:1:0	25	12.6
R4/2-4	5-6	9:1:0	50	3.2
R4/2-5	7-10	9:1:0	100	4.2
R4/2-6	11-16	9:1:0	150	10.7
R4/2-7	17-20	9:1:0	100	79.3
R4/2-8	21-22	9:1:0.5	50	144.9
R4/2-9	23	9:1:0.5	25	57.1
R4/2-10	24-28	0:0:1	125	166.7
R4/2-11	29-32	0:0:1	100	232

Fraction R4/2-7, after eluted with EtOAc, gave white precipitate (34.2 mg) and recrystallized from DMSO to furnish a new colorless amorphous powder (R4/2-7, rostratin F) (Scheme 3.5). Biological activities and structure elucidation of this compound were described in Chapter IV.

In addition, fraction R5 was further separated on a silica gel flash column (2.5 x 16 cm), using EtOAc-Hexane (9:1), EtOAc-Hexane-MeOH (9:1:0.5), and MeOH as the

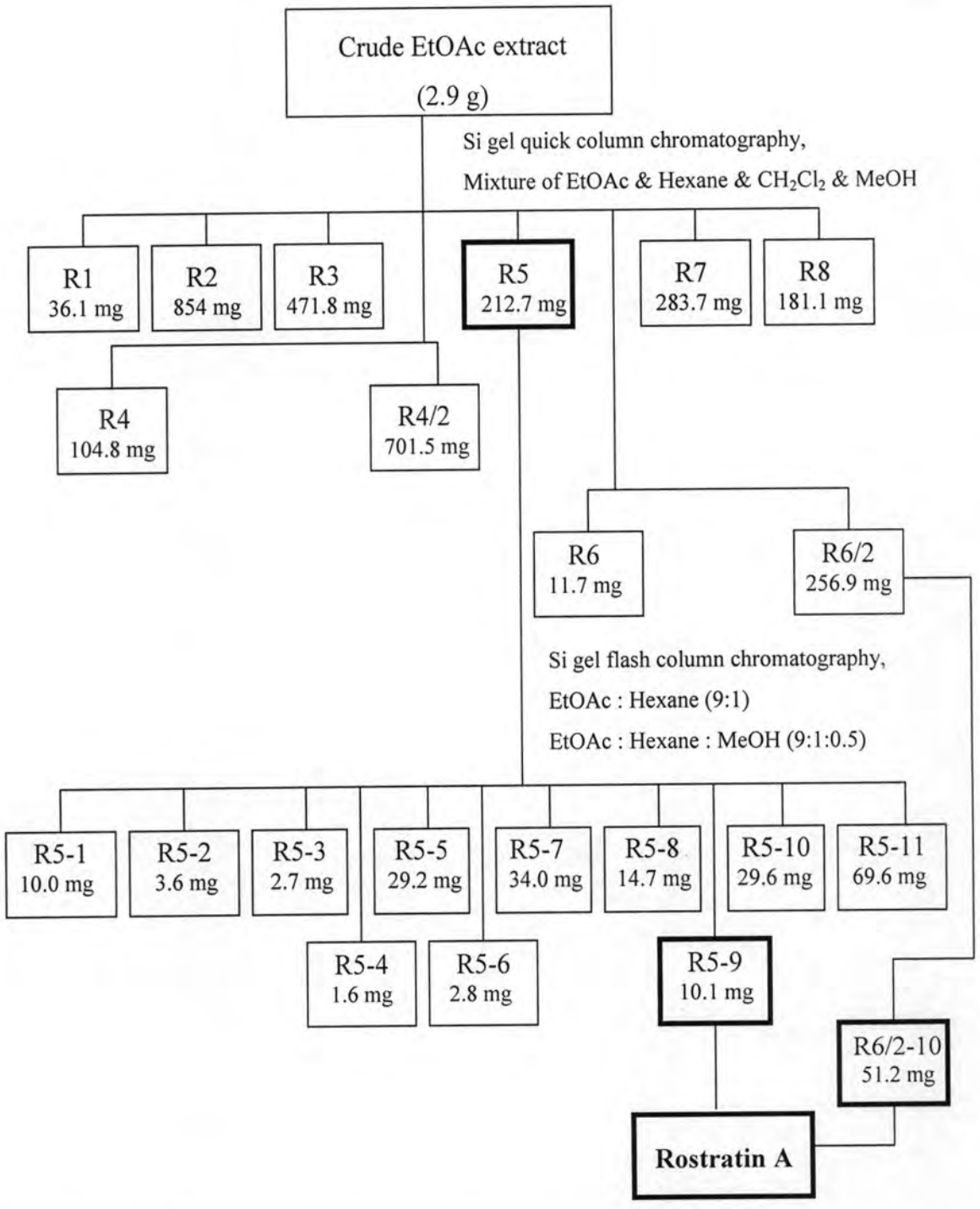


Scheme 3.5 Isolation of compound R4/2-7 (exserohilin B) from *Exserohilum rostratum* RNAS5

eluting solvent, respectively. Fractions (25 ml each) were collected, and examined. Fraction combination was guided by TLC on silica gel plate with EtOAc and Hexane (9:1) as the developing solvent. Fractions with similar TLC patterns were pooled and dried. Eleven fractions (R5-1-11) were obtained (Table 3.9).

Table 3.9 Isolation of compound **R5-9** from *Exserohilum rostratum* RNAS5 by a silica gel flash column

Fraction code	Number of fractions	Ratio of EtOAc:Hexane:MeOH	Volume (ml) of eluent	Weight (mg)
R5-1	1-4	9:1:0	100	10.0
R5-2	5-6	9:1:0	50	3.6
R5-3	7-9	9:1:0	75	2.7
R5-4	10	9:1:0	25	1.6
R5-5	11-16	9:1:0	150	29.2
R5-6	17	9:1:0	25	2.8
R5-7	18-21	9:1:0	100	34
	22-24	9:1:0.5	75	
R5-8	25	9:1:0.5	25	14.7
R5-9	26	9:1:0.5	25	10.1
R5-10	27-29	0:0:1	75	29.6
R5-11	30-32	0:0:1	75	69.6



Scheme 3.6 Isolation of compound R5-9 (rostratin A) from *Exserohilum rostratum* RNAS5

Fraction R5-9 (10.1 mg) was crystallized from a mixture of CH_2Cl_2, MeOH and EtOAc to give the yellow needles which was later identified as the known compound, rostratin A (Scheme 3.6). Moreover, fraction R6/2 was also fractionated and yielded fraction R6/2-10 (51.2 mg) which was found to be R5-9, rostratin A (total 61.3 mg). Biological activities and structure elucidation of this compound were described in Chapter IV.

The other fractions obtained from the strain *Exserohilum rostratum* RNAS5 were kept in the desiccator for further investigation.

4. Physical and chemical properties of the isolated compounds

4.1 Rostratin A (compound R5-9)

UV : λ_{max} nm, in methanol; Figure 4.9

207.2 nm

HREIMS : *m/z* 364.1652 (fragment ion)

m/z 428 (molecular ion)

^{1}H-NMR : δ_H (ppm), 500 MHz, in pyridine-d_6

See Figure 4.10 and Table 4.1 (Part B, Section 3.1, Chapter IV)

^{13}C-NMR : δ_c (ppm), 125 MHz, in pyridine-d_6

See Figure 4.11 and Table 4.1 (Part B, Section 3.1, Chapter IV)

4.2 Exserohilin A (compound W3)

UV : λ_{max} nm, in methanol; Figure 4.25

207.2 nm

IR : ν_{max} cm^{-1}; Figure 4.24

3,354, 1682

HREIMS : *m/z* 422.0637 (found)

^{1}H-NMR : δ_H (ppm), 500 MHz, in pyridine-d_6

See Figure 4.26 and Table 4.3 (Part B, Section 3.2, Chapter IV)

^{13}C-NMR : δ_c (ppm), 125 MHz, in pyridine-d_6

See Figure 4.27 and Table 4.3 (Part B, Section 3.2, Chapter IV)

4.3 Exserohilin B (compound R4/2-7)

UV : λ_{max} nm, in methanol; Figure 4.38

207.2 nm

HREIMS : *m/z* 454.0307 (found)

^{1}H-NMR : δ_H (ppm), 500 MHz, in pyridine-d_6

See Figure 4.39 and Table 4.4 (Part B, Section 3.3, Chapter IV)

^{13}C-NMR : δ_c (ppm), 125 MHz, in pyridine-d_6

See Figure 4.40 and Table 4.4 (Part B, Section 3.3, Chapter IV)