CHAPTER III

EXPERIMENTAL

1. Source of Plant Material

The wood of *Artocarpus altilis* (Park.) Fosb. was collected from Tahlingchan, Bangkok, Thailand in June, 1993. The plant material was identified by Mrs. Nuchattra Chansuwanit of the Botanical Section, Division of Medicinal Plant Research and Development, Department of Medical Science, Ministry of Public Health, Nonthaburi, Thailand.

2. General Techniques

2.1 Thin-layer Chromatography (TLC)

Technique : One way, ascending

Adsorbent : Silica gel 60 F_{254} (E. Merck) precoated plate

Layer thickness : 0.2 mm

Distance : 5 cm

Temperature : Laboratory temperature (30-35°C)

Detection : 1. Ultraviolet light at the wavelength of 254 and 365 nm

: 2. 10% Sulfuric acid in ethanol

2.2 Column Chromatography

2.2.1 Quick Column Chromatography

Adsorbent : Silicagel 60 (No. 9385) particle size 0.040-0.063 nm (230-400 mesh ASTM) (E. Merck)

Packing method : Dry packing

Sample loading : The sample was dissolved in a small amount of organic solvent, mixed with a small quantity of adsorbent, triturated, then dried and

added gently to the top of the column.

Examination of eluates : Fractions were examined by TLC observing under ultraviolet light at the wavelength of 254 and 365 nm and followed by spraying with 10% sulfuric acid in ethanol before being heated at 105 °C for 10 min. Those fractions of similar pattern were combined.

2.2.2 Flash Column Chromatography

Adsorbent : Silica gel 60 (No. 9385) particle size 0.040-0.063 mm (230-400 mesh ASTM) (E. Merck)

Packing method : Dry packing

Sample loading : The sample was dissolved in a small amount of organic solvent, mixed with a small quantity of adsorbent, triturated, then dried and added gently to the top of the column.

Examination of eluates : Fractions were examined in the same manner as described in the section 2.2.1

2.2.3 Gel Filtration Chromatography

Gel filter : Sephadex LH-20 (Pharmacia)

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

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

Examination of eluates : Fractions were examined in the same manner as described in section 2.2.1

2.3 Spectroscopy

2.3.1 Ultraviolet (UV) Absorption Spectra

The spectra were obtained on a Jasco Uvidec-650 double beam spectrophotometer (Division of Medicinal Plant Research and Development, Department of Medical Sciences, Ministry of Public Health).

2.3.2 Infrared (IR) Absorption Spectra

The spectra were obtained from a Perkin Elmer FT-IR spectrometer 1760x (Scientific and Technological Research Equipment Center, Chulalongkorn University) in potassium bromide disc and as a solution in acetone.

2.3.3 Mass Spectra (MS)

The Electron Impact Mass Spectra (EIMS) were performed on a Finnigan MAT Incos 50 mass spectrometer (Department of Chemistry, Faculty of Science, Mahidol University).

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

The 500 MHz ^{1}H NMR and 125 MHz ^{13}C NMR spectra were obtained with JEOL JMN-A 500 spectrometer (Scientific and Technological Research Equipment Center, Chulalongkorn University).

The solvent for NMR spectra was deuterated acetone (CD_3COCD_3). The chemical shifts were reported in ppm scale using the chemical shift of tetramethylsilane (TMS) at 0 ppm as the reference signal.

2.4 Solvents

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

3. Extraction Procedure

The dried coarsely powdered wood of *Artocarpus altilis* (Park.) Fosb. (4 kg) was repeatedly macerated for three 3-day periods with 95% ethanol and then filtered. The filtrates from each maceration were pooled and concentrated under reduced pressure at temperature not exceeding 45°C. The initial extract, approximately 250 ml. was partitioned with chloroform (850 ml). The chloroform fraction was dried with Na_2SO_4, then evaporated to dryness under reduced pressure to give 28.20 g of brown syrupy mass at the yield of 0.71 % based on dried weight of *A. altilis* 's powder. The syrupy mass was further partitioned with hexane and methanol. Both hexane and methanol extract were separately evaporated to dryness under reduced pressure to yield 15.72 g and 12.48 g (0.39 % and 0.31 % based on dried weight), respectively.

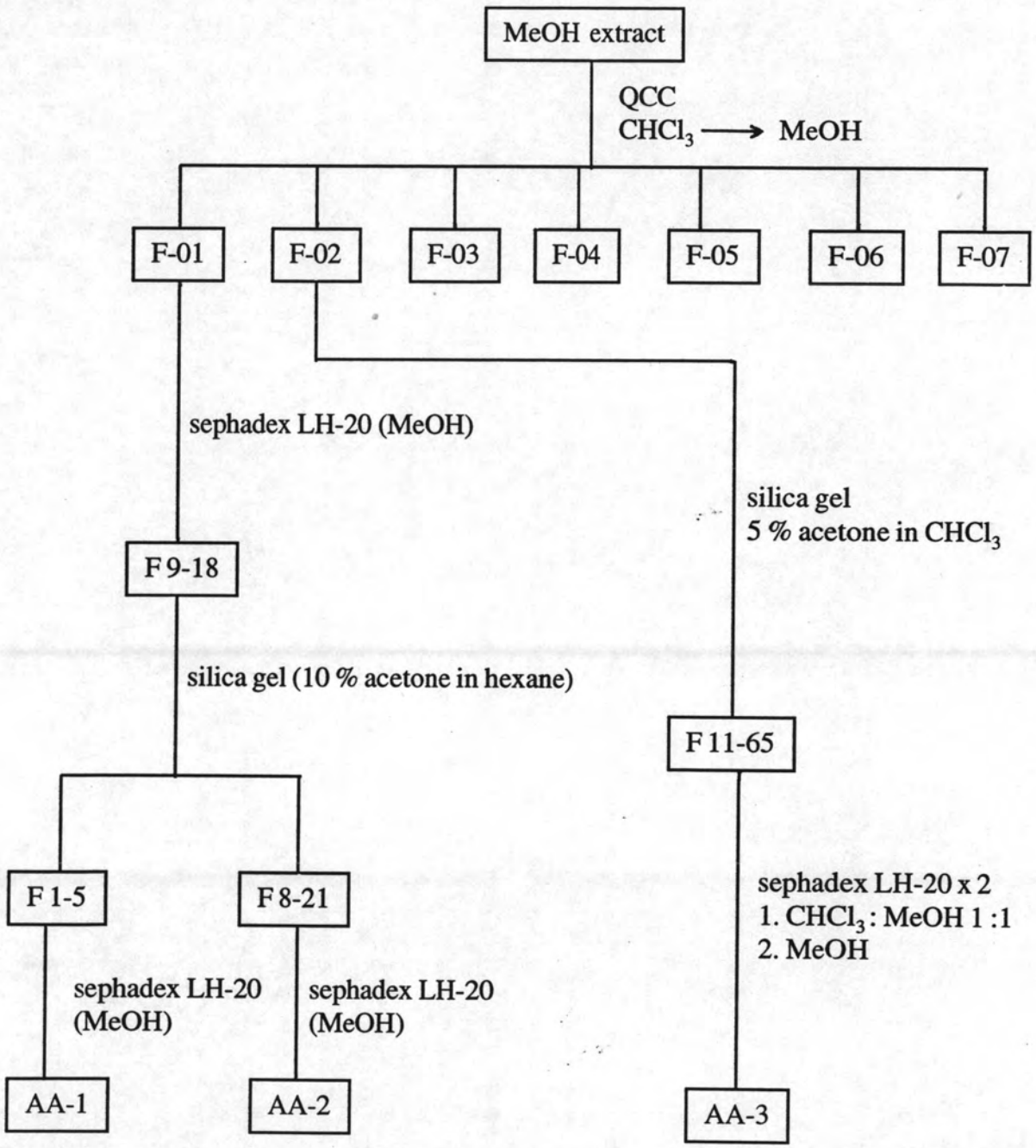
The water extract was partitioned with butanol and evaporated to dryness under reduced pressure to yield 38.87 g of butanol extract and 71 g of water extract (0.97 % and 1.78 % based on dried weight, respectively).

4. Isolation Procedure

4.1 The Isolation of AA-1

Methanol extract (12.48 g) was dissolved in a small volume of the chloroform/methanol mixture, triturated with silica gel 60 (No. 9385) and dried under reduced pressure. It was then fractionated by quick column chromatographic technique using a sintered glass filter column of silica gel (7 × 3.5 cm). The eluents were used in the order as shown below :

-chloroform	1840 ml	fraction #	1-23
-chloroform : methanol (95 : 5)	2560 ml	fraction #	24-55
-chloroform : methanol (9 : 1)	960 ml	fraction #	56-67
-chloroform : methanol (1 : 1)	160 ml	fraction #	68-69
-methanol			



Scheme 7 Isolation scheme of *A. altilis* from methanol extract

Methanol was used to wash the column until the eluates were diluted and clear compaired to previous ones. The eluates were examined by TLC using 20% chloroform in hexane and 10% methanol in chloroform as developing solvents. Fractions with similar chromatographic pattern were combined.

Table 10. The combined fractions from crude methanol extract

Fractions	Number of eluates	Weight (g)
F-01	1-8	0.37
F-02	9-25	3.47
F-03	26-39	3.53
F-04	40-58	2.87
F-05	59-67	1.25
F-06	68-69	1.94
F-07	methanolwashed	0.07

Fraction F-01 (0.37 g) was divided into 90-mg portions. Each was fractionated by gel filtration chromatography using a column of Sephadex LH-20 (100 g, 2 × 70 cm) with methanol as eluent. Twenty fractions, approximately 20 ml each were collected based on the colour bands. The eluates were examined by TLC and fractions giving the same chromatographic pattern were combined.

The TLC chromatogram of fractions 9-18 indicated the presence of two yellow compounds. The isolation of each compound was carried out.

These fractions (0.30 g)were combined and then equally divided into 2 portions, each was fractionated by column chromatography using siliga gel 60 (2.5 × 20 cm). The eluent was 10% acetone in hexane. Thirty-ml fractions were collected based on the colour band. The eluates were examined by TLC and fractions showing the same pattern were combined.

Fractions number 1-5 showed one yellow spot (other fractions showing another yellow spot was later purified according to the procedure described in section 4.2). This was further purified by means of gel filtration chromatography with Sephadex LH-20 column (2 × 70 cm), using methanol as eluent. The eluates, approximately 15 ml each were collected depending on the colour band.

The yellow compound was obtained from fractions 3-5 as amorphous powder (14.30 mg). The yield was 0.36×10^{-3} % based on dried weight. This compound, codenamed AA-1 was identified as isocyclomorusin (compound no. 51).

4.2 The Isolation of AA-2

The other fractions of F-01 (number 8-21 achieved from silica gel column), which also showed another yellow spot in TLC chromatogram, was further purified using a column of Sephadex LH-20 (2 × 70 cm) with methanol as eluent. The 10 fraction eluates were collected depending on colour band (approximately 20 ml per fraction). A yellow compound was obtained as amorphous powder from fractions 4-7. It was recrystallized from acetone as 200.39 mg pale yellow needles, codenamed AA-2. It yielded 5.01×10^{-3} % based on dried weight. This compound was later identified as cycloartocarpin (compound no.31).

4.3 The Isolation of AA-3

Fraction F-02 (3.47 g) was dissolved in a small volume of acetone-chloroform mixture, triturated with 6 g of silica gel and dried under reduced pressure. It was fractionated by column chromatographic technique using a silica gel column (3 × 15 cm) with 5 % acetone in chloroform as eluent. Twenty-ml fractions were collected and combined after examining with TLC, using chloroform : methanol (95 :5) as developing solvent.

Fractions 11-65 (1.49 g) were combined and further purified by using a column of Sephadex LH-20 (2 × 75 cm) with the mixture of chloroform and methanol (1 : 1) as eluent. Fifteen-ml fractions were collected based on the colour band. The fractions containing yellow compound were again combined and purified by means of a column of Sephadex LH-20 (2 × 70 cm) with methanol as eluent. Fifteen-ml fractions were collected and examined.

A yellow prism was obtained from eluate no. 5-7. Its total amount was 189.0 mg (4.72×10^{-3} % yielded based on dried weight) and was named AA-3. This compound was identified as artocarpin (compound no. 6).

5. Characterization of Isolated Compounds

5.1 Characterization of AA-1

AA-1 was obtained as yellow amorphous powder (14.30 mg). It was soluble in chloroform and acetone.

EIMS : *m/z* (% relative intensity); Figure 2

418 (48), 403 (100), 363 (30), 348(6), 347 (29), 319 (3)281 (2), 279 (1), 256 (1), 203 (17), 194 (15), 174 (11), 149 (6), 137 (4), 115 (2), 73 (4), 44 (8)

IR : υ cm^{-1}, in acetone; Figure 3

3508-3400, 3056, 2971, 1655, 1623, 1556, 1467, 1260, 1187

UV : λ_{max} nm (log ε), in methanol; Figure 4

371 (3.95), 308 (3.45)

λ_{max} nm, in methanol with $AlCl_3$; Figure 4

384, 311

λ_{max} nm, in methanol with $AlCl_3$/HCl; Figure 4

385, 312

^{1}H-NMR : δ ppm, 500 MHz, in acetone-d_6; Figure 5-7

1.45 (s), 1.67 (d, *J* = 1.2 Hz), 1.93 (d, *J* = 1.2 Hz), 5.47 (br d, *J* =9.5 Hz), 5.75 (d, *J* = 10.1 Hz), 6.19 (d, *J* = 9.5 Hz), 6.42 (d, *J* = 2.4 Hz),

6.45 (s), 6.63 (overlapping dd, J = 8.5, 2.4 Hz), 6.64 (d, J = 10.1 Hz), 7.69 (d, J = 8.6 Hz), 13.26 (br.s)

^{13}C-NMR : δ ppm, 125 MHz, in acetone-d_6; Figure 8

18.6, 25.8, 28.3, 70.2, 78.7, 95.7, 104.8, 106.0, 106.1, 108.3, 109.9, 110.8, 115.7, 122.0, 126.3, 129.3, 138.8, 156.6, 157.2, 157.3, 159.1, 159.9, 164.1, 179.2

melting point 245-246 ° C (uncorrected)

5.2 Characterization of AA-2

AA-2 was obtained as yellow needles from acetone (200.39 mg). It was soluble in acetone.

EIMS : *m/z* (% relative intensity); Figure 23

434 (35), 419 (2), 391 (21), 379 (25), 363 (21),351 (1), 335 100), 309 (7), 233 (1), 189 (11),162 (5), 137 (5), 115 (4), 91 (4), 69 (6),55 (3)

IR : υ cm^{-1}, KBr disc; Figure 24

3309, 2936, 2864, 1651, 1622, 1583, 1553, 1480, 1211, 1148, 1133, 810

UV : λ_{max} nm (log ε), in methanol; Figure 25

368 (4.43), 303 (3.90)

λ_{max} nm, in methanol with $AlCl_3$; Figure 25

375, 305

λ_{max} nm, in methanol with $AlCl_3$/HCl; Figure 25

376, 305

^{1}H-NMR : δ ppm, 500 MHz, in acetone-d_6; Figure 26-28

1.07 (s), 1.95 (d, J = 0.9 Hz), 2.03 (d, J = 0.9 Hz), 2.42 (m), 3.98 (s), 5.46 (d, J = 9.5 Hz), 6.22 (d, J = 9.5 Hz), 6.42 (d, J = 2.1 Hz), 6.57 (d, J = 16.2 Hz), 6.61 (dd, J = 8.6, 2.1 Hz), 6.67 (dd, J =16.2, 7.02 Hz), 6.74 (s), 7.71 (d, J = 8.6 Hz), 9.32 (s), 13.63 (s)

^{13}C-NMR : δ ppm, 125 MHz, in acetone-d_6; Figure 29

18.6, 23.0, 25.8, 33.9, 56.6, 70.4, 91.0, 104.9, 105.9, 108.3, 110.1, 110.4, 110.8, 116.8, 122.1, 126.2, 138.9, 142.5, 156.2, 156.5, 159.1, 159.8, 163.6, 164.0, 179.3

melting point 263-265 °C (uncorrected)

5.3 Characterization of AA-3

AA-3 was obtained as yellow prism from methanol (189.0 mg). It was soluble in acetone.

EIMS : *m/z* (% relative intensity); Figure 42

436 (59), 421 (5), 393 (100), 381 (22), 379 (25), 365 (12), 349 (5), 337 (44), 322 (5), 295 (5), 233 (4), 203 (4), 202 (2), 179 (12), 147 (16), 137 (7), 91 (16), 77 (13), 69 (23), 55 (11)

IR : υ cm^{-1}, KBr disc; Figure 43

3415-3335, 2958, 2927, 1651, 1620, 1451, 1351, 1206, 1146, 977, 809,

UV : λ_{max} nm (logε), in methanol; Figure 44

324 (4.05), 265 sh (4.19)

λ_{max} nm, in methanol with $AlCl_3$; Figure 44

327, 265 sh

λ_{max} nm, in methanol with $AlCl_3$/HCl; Figure 44

327, 265 sh

^{1}H-NMR : δ ppm, 500 MHz, in acetone-d_6; Figure 45-47

1.07 (s), 1.08 (s), 1.42 (s), 1.55 (s), 2.43 (m), 3.11 (d, J = 7.0 Hz),3.95 (s), 5.12 (br t, J =7.0 Hz), 6.51 (dd, J = 8.2, 2.1m Hz), 6.53 (s), 6.56 (d, J = 2.1 Hz), 6.59 (dd, J =16.2, 0.9 Hz), 6.72 (dd, J = 16.2, 7.3 Hz), 7.20 (d, J = 8.2 Hz), 8.76 (br. s), 13.94 (s)

^{13}C-NMR : δ ppm, 125 MHz, in acetone-d_6; Figure 48

17.6, 23.1, 24.6, 25.7, 33.9, 56.5, 90.5, 103.8, 105.5, 108.0, 109.7, 112.8, 117.0, 121.9, 122.5, 132.2, 132.3, 142.2, 157.2, 157.4, 159.8, 161.5, 162.4, 163.8, 183.3

melting point 186-187 °C (uncorrected)