

## CHAPTER III

### EXPERIMENTAL

#### 1. Source of Plant Material

The leaves of *Artocarpus gomezianus* Wall. ex Trec. were obtained from Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand in November, 1993. The plant material was authenticated by comparison with the herbarium specimen in the Botany Section, Technical Division, Department of Agriculture, Ministry of Agriculture and Cooperative. A voucher specimen was deposited in the herbarium of the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

#### 2. General Techniques

##### 2.1 Analytical Thin-layer Chromatography (TLC)

- Technique** : One way, ascending  
**Absorbent** : Silica gel 60 F254 (E. Merk) precoated plate  
**Layer thickness**: 0.2 mm  
**Distance** : 5.0 cm  
**Temperature** : Laboratory temperature (30-35°C)  
**Detection** : 1. Visual detection under ultraviolet light at the wavelength of 254 and 365 nm  
2. Anisaldehyde-sulphuric acid spraying reagent ( 0.5% ethanolic solution of anisaldehyde with 5% sulphuric acid)

##### 2.2 Column Chromatography (CC)

- Absorbent** : Silica gel 60 (No. 7734) particle size 0.063-0.200 nm  
Silica gel 60 (No. 9385) particle size 0.040-0.063 nm  
**Packing method**: Dry packing  
**Sample loading** : A portion of crude extract was dissolved in a small amount of organic solvent, mixed with a small quantity of adsorbent, then dried, triturated and added gently on the column.  
**Examination of eluates** : Fractions were examined by TLC using visual detection under ultraviolet light at wavelength of 254 and 365 nm and sprayed with anisaldehyde-sulphuric acid spraying reagent.

## **2.3 Spectroscopy**

### **2.3.1 Ultraviolet (UV) Absorption Spectra**

The spectra were obtained on Milton Roy Spectronic 3000Array spectrometer (Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

### **2.3.2 Infrared (IR) Absorption Spectra**

The spectra were obtained from a Shimadzu IR-440 infrared spectrometer (The Scientific and Technological Research Equipment Center, Chulalongkorn University) in potassium bromide discs.

### **2.3.3 Mass Spectra (MS)**

The Electron Impact Mass Spectra (EIMS) were obtained by operating at 70 eV with a Fisons VG Trio 2000 quadrupole mass spectrometer (Department of Chemistry, Faculty of Science, Chulalongkorn University) and the High Resolution Fast-Atom Bombardment Mass Spectra (HR-FAB-MS) were measured with a Hitachi RMU-7M mass spectrometer.

### **2.3.4 Proton and Carbon-13 Nuclear Magnetic Resonance ( $^1\text{H}$ and $^{13}\text{C}$ NMR) Spectra**

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

The operated solvent for NMR spectra were deuterated chloroform ( $\text{CDCl}_3$ ), deuterated dimethylsulfoxide ( $\text{DMSO-d}_6$ ). 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 commercial grade and had to be redistilled prior to use.

## **3. The Extraction**

The dried powder of the leaves of *Artocarpus gomezianus* Wall. ex Trec. (1.8 kg) was macerated twice over a period of three days, with 95% ethanol and then filtered. The filtrate of each maceration was concentrated to remove ethanol under reduced pressure to yield 146 g of syrupy mass.

#### 4. The Isolation

##### 4.1. The Isolation of AG-1 and AG-2

The syrupy mass (146 g) was dissolved in a small volume of chloroform/methanol mixture, triturated with silica gel 60 (No. 7734) and dried under reduced pressure. It, then, was fractionated by quick column chromatographic technique using a sintered glass filter column of silica gel (20x4 cm). The eluents were used in the order as shown below :

- hexane	3,300 ml	fraction # 1-11
- chloroform in hexane (5%)	1,800 ml	fraction # 12-17
- chloroform in hexane (10%)	900 ml	fraction # 18-20
- chloroform in hexane (15%)	3,000 ml	fraction # 21-30
- chloroform in hexane (20%)	3,000 ml	fraction # 31-40
- chloroform in hexane (30%)	600 ml	fraction # 41-42
- chloroform in hexane (40%)	1,200 ml	fraction # 43-46
- chloroform in hexane (50%)	1,500 ml	fraction # 47-51
- chloroform in hexane (60%)	3,600 ml	fraction # 52-68
- chloroform in hexane (70%)	3,900 ml	fraction # 69-81
- chloroform	9,300 ml	fraction # 82-112
- methanol in chloroform (5%)	5,700 ml	fraction # 113-131
- methanol in chloroform (10%)	1,500 ml	fraction # 132-136
- methanol in chloroform (20%)	600 ml	fraction # 137-138
- methanol in chloroform (30%)	1,200 ml	fraction # 139-142
- methanol in chloroform (40%)	600 ml	fraction # 143-144
- methanol in chloroform (50%)	300 ml	fraction # 145

Methanol was used to wash the column until the eluates were diluted and clear comparing to former ones.

The fractional volume was about 300 ml and examined by TLC using 20% chloroform in hexane and 10% methanol in chloroform as developing solvents. Fractions giving similar chromatographic pattern were combined and designated.

**Table 8** The combined fractions from crude extract

Fraction	Number of Eluates
F-01	1-16
F-02	17-28
F-03	29-48
F-04	49-54
F-05	55-61
F-06	62-78
F-07	79-98
F-08	99-141
F-09	142-145
F-10	MeOH eluted

From F-06, the white amorphous compound was precipitated. It was recrystallized from chloroform as white flakes. It yielded 156 mg ( $8.67 \times 10^{-3}$  % based on dry weight) and was named as AG-1. This compound was identified as 1-dotriacontanol.

From F-07, the white compound was crystallized. It was recrystallized from hexane as colorless needles. It yielded 72 mg ( $4.00 \times 10^{-3}$  % based on dry weight) and was named as AG-2. This compound was identified as  $\beta$ -sitosterol.

#### **4.2 The Isolation of AG-3**

Fraction F-04 (2.6 g) was dissolved in a small volume of chloroform and triturated with silica gel 60 (No. 7734) (5 g). This mixture was dried under reduced pressure. It was fractionated by the column chromatographic technique using a column of silica gel (4.5x25 cm) with a gradient system of 1-50 % chloroform in hexane as an eluent. Forty ml fractions were collected based on the color band. The eluates were examined by TLC using chloroform : hexane (4:6) as developing solvent. The fractions showing the same pattern were combined.

The colorless needles were crystallized from fractions 144-158. It yielded 69 mg ( $3.83 \times 10^{-3}$  % based on dry weight), was named as AG-3, and was identified as lupiol-3-acetate.

### 4.3 The Isolation of AG-4

Fraction F-05 (2.4 g) was dissolved in a small volume of chloroform, triturated with silica gel 60 (No. 7734) (5 g) and dried under reduced pressure. It was fractionated by the column chromatographic technique using a silica gel column (4.5x15 cm) with a gradient of chloroform in hexane, as eluent. Forty ml fractions were collected and combined after examining with TLC using chloroform as developing solvent. The results were shown in Table 9.

**Table 9** Solvent systems used in column chromatography of fraction F-05

Fraction	Eluent
1-14	chloroform:hexane (1:4)
15-90	chloroform:hexane (1:4)
91-111	chloroform:hexane (1:4)
112-147	chloroform:hexane (2:3)
148-198	chloroform:hexane (2:3), (1:1)

Fractions 148-198 (731 mg) were combined and further separated using a column of silica gel 60 (No. 9385) (2.5x40 cm) with hexane : ethylacetate (49:1) as an eluent. Fifty ml fractions were collected and combined after examining with TLC using developing solvent similar to an eluent. The eluates No. 13-15 showed one spot on TLC. The colorless needles was crystallized from hexane. It yielded 25 mg ( $1.39 \times 10^{-3}\%$  based on dry weight), was named as AG-4, and was identified as simiarenol.

### 4.4 The Isolation of AG-5

Fraction F-09 (22 g) was isolated by quick column chromatographic technique using a sintered glass filter column of silica gel 60 (No. 9385) (11x4 cm). The eluents were used in the order as shown below.

-chloroform	300 ml	fraction # 1
-ethylacetate in chloroform	600 ml	fraction # 2-3
-ethylacetate	1,500 ml	fraction # 4-8
-methanol in ethylacetate (5 %)	1,200 ml	fraction # 9-11
-methanol in ethylacetate (10 %)	2,100 ml	fraction # 12-18

The fractional volume was about 300 ml and examined by TLC using 10% methanol in ethylacetate as developing solvent. Fractions giving similar chromatographic pattern were combined. The eluates No. 12-13 were dried under reduced pressure and then a white compound was precipitated. It was recrystallized from mixture of ethylacetate and methanol as colorless needles. It yielded 3.02 g ( $1.68 \times 10^{-1}\%$  based on dry weight), was named as AG-5, and was identified as arbutin.

## 5. Characterization of the Isolated Compounds

### 5.1 Characterization of AG-1

AG-1 was obtained as white crystals. It was soluble in chloroform.

<b>EIMS ;</b>	<i>m/z</i> (% relative intensity); Figure 2				
	449 (0.37),	421 (0.26),	393 (7),	365 (7),	364 (3),
	209 (2),	195 (3),	181 (3),	167 (4),	153 (6),
	139 (9),	125 (18),	111 (36),	97 (66),	83 (72),
	69 (67),	57 (100),	55 (64),	43 (100)	
<b>IR ;</b>	$\nu$ cm <sup>-1</sup> , KBr disc; Figure 3				
	3424, 3298, 2918, 2849, 1473, 1463, 1062, 720				
<b><sup>1</sup>H NMR ;</b>	$\delta$ ppm, 500 MHz, in chloroform-d; Figure 4				
	0.88 (t, <i>J</i> =7.2 Hz), 1.25 (br s), 1.55 (m), 3.63 (t, <i>J</i> =7.2 Hz)				
<b><sup>13</sup>C NMR ;</b>	$\delta$ ppm, 125 MHz, in chloroform-d; Figure 5				
	14.06, 22.65, 25.71, 29.31, 29.39, 29.55, 29.65, 31.87, 32.78, 63.07				

### 5.2 Characterization of AG-2

AG-2 was crystallized as colorless needles from hexane. It was soluble in chloroform.

<b>EIMS ;</b>	<i>m/z</i> (% relative intensity); Figure 6				
	414 (32),	399 (14)	396 (17)	381 (14),	329 (21),
	273 (15),	255 (22),	231 (16),	213 (29),	173 (15),
	163 (22),	161 (24),	159 (28),	147 (24),	145 (39),
	135 (22),	133 (29),	131 (20),	121 (23),	119 (23),
	109 (23),	107 (43),	105 (37),	95 (51),	83 (86),
	81 (57),	69 (100),	57 (94)		
<b>IR ;</b>	$\nu$ cm <sup>-1</sup> , KBr disc; Figure 7				
	3500-3200, 2960-2860, 1642, 1465, 1381, 1062, 840, 802				
<b><sup>1</sup>H NMR ;</b>	$\delta$ ppm, 500 MHz, in chloroform-d; Figure 8-9				
	0.67-2.3, 3.52 (m), 5.34 (m)				
<b><sup>13</sup>C NMR ;</b>	$\delta$ ppm, 125 MHz, in chloroform-d; Figure 10-11				
	11.87, 11.99, 18.78, 19.05, 19.40, 19.82, 21.10, 23.08, 24.31, 26.09, 28.25, 29.16, 31.67, 31.91, 33.95, 36.15, 36.51, 37.27, 39.78, 42.31, 45.84, 50.41, 56.78, 71.80, 121.71, 140.77				

### 5.3 Characterization of AG-3

AG-3 was obtained as colorless needles from chloroform. It was soluble in chloroform and ethylacetate, and insoluble in acetone and ethanol.

<b>EIMS ;</b>	<i>m/z</i> (% relative intensity); Figure 12				
	468 (7),	455 (3),	408 (6),	297 (6),	257 (7),
	229 (17),	218 (78),	203 (39)	189 (98),	175 (26),
	161 (29),	147 (45),	135 (100),	121 (51)	107 (65),
	95 (70),	81 (83),	69 (79),	55 (79)	
<b>IR ;</b>	$\nu$ cm <sup>-1</sup> , KBr disc; Figure 13				
	3073, 2941, 2872, 2854, 1736, 1640, 1455, 1367, 1247, 1025, 980, 877				
<b><sup>1</sup>H NMR ;</b>	$\delta$ ppm, 500 MHz, in chloroform-d; Figure 14-15				
	0.79-1.03, 1.68 (s), 2.04 (s), 4.47 (m), 4.57 (br s), 4.69 (br s)				
<b><sup>13</sup>C NMR ;</b>	$\delta$ ppm, 125 MHz, in chloroform-d; Figure 16				
	14.50, 15.97, 16.16, 16.48, 17.99, 18.20, 19.27, 20.93, 21.30, 23.70, 25.10, 27.43, 27.94, 29.83, 34.21, 35.56, 37.07, 37.78, 38.04, 38.39, 39.98, 40.84, 42.81, 42.98, 48.00, 48.29, 50.35, 55.38, 80.96, 109.34, 105.93, 170.97				

#### 5.4 Characterization of AG-4

AG-4 was obtained as colorless needles from hexane. It was soluble in hexane and chloroform.

<b>EIMS ;</b>	<i>m/z</i> (% relative intensity); Figure 17				
	426 (4),	411 (2),	408 (3),	274 (100),	259 (90),
	245 (13),	231 (26),	205 (13),	175 (75),	152 (42),
	134 (66),	122 (56),	107 (40),	95 (50),	81 (40),
	69 (39),	55 (37)			
<b>IR ;</b>	$\nu$ cm <sup>-1</sup> , KBr disc; Figure 18				
	3508, 2931, 2868, 1650, 1470, 1545, 1384, 1052, 831, 818				
<b><sup>1</sup>H NMR ;</b>	$\delta$ ppm, 500 MHz, in chloroform-d; Figure 19-20				
	0.782 (3H,s),	0.829 (3H, d, <i>J</i> =6.7 Hz),	0.888 (3H, d, <i>J</i> =6.7 Hz),		
	0.895 (3H, s),	0.926 (3H, s),	1.006 (3H,s),		
	1.045 (3H, s),	1.140 (3H, s),	1.16-2.16,		
	3.467 (1H,br s),	5.615 (1H, ddd, <i>J</i> =1.93, 1.93, 5.81)			
<b><sup>13</sup>C NMR ;</b>	$\delta$ ppm, 125 MHz, in chloroform-d; Figure 16				
	15.00, 15.74, 16.06, 17.84, 18.06, 19.91, 21.95, 22.89, 24.06, 25.46, 27.78, 28.30, 29.00, 29.06, 29.11, 30.77, 34.14, 34.82, 35.41, 38.60, 39.31, 40.81, 42.80, 44.26, 50.25, 51.73, 60.04, 76.34, 121.96, 142.00				

#### 5.5 Characterization of AG-5

AG-5 was obtained as colorless needles from mixture of ethylacetate and methanol. It was soluble in methanol.

- HR-FAB-MS** (dithiodiethanol + NaCl) ;  $m/z$  (% relative intensity); Figure 23  
295 (29), 273 (16)
- EIMS** ;  $m/z$  (% relative intensity); Figure 24  
110 (100)
- UV** ;  $\lambda_{\max}$  nm (log  $\epsilon$ ), in methanol ; Figure 25  
224 (log  $\epsilon$  3.82), 286 (log  $\epsilon$  3.32)
- IR** ;  $\nu$  cm<sup>-1</sup>, KBr disc; Figure 26  
3372, 3292, 3204, 1611, 1513, 1461, 1223, 1106, 1080, 1050, 1027,  
1016, 833
- <sup>1</sup>H NMR** ;  $\delta$  ppm, 500 MHz, in dimethylsulfoxide-d<sub>6</sub>; Figure 27-28  
3.13 (1H, ddd,  $J=9.21, 8.55, 5.26$  Hz),  
3.17 (1H, ddd,  $J=9.21, 7.24, 5.26$  Hz), 3.20-3.25 (2H, overlapping),  
3.45 (1H, ddd,  $J=11.84, 5.26, 5.26$  Hz),  
3.67 (1H, ddd,  $J=11.84, 5.26, 0.97$  Hz), 4.51 (1H, t,  $J=5.26$  Hz),  
4.62 (1H, d,  $J=7.24$  Hz), 4.94 (1H, d,  $J=5.26$  Hz),  
5.00 (1H, d,  $J=5.26$  Hz), 5.20 (1H, d,  $J=5.26$  Hz),  
6.65 (2H, d,  $J=8.55$  Hz), 6.85 (2H, d,  $J=8.55$  Hz),  
8.97 (1H, s)
- <sup>1</sup>H NMR** ;  $\delta$  ppm, 500 MHz, in pyridine-d<sub>5</sub>  
4.075 (1H, ddd,  $J=9.38, 5.00, 2.50$  Hz), 4.27-4.39 (3H, m),  
4.40 (1H, dd,  $J=11.84, 5.26$  Hz), 4.54 (1H, dd,  $J=11.84, 2.63$  Hz),  
5.517 (1H, d,  $J=7.62$  Hz), 7.11 (2H, d,  $J=8.33$  Hz),  
7.38 (2H, d,  $J=8.33$  Hz)
- <sup>13</sup>C NMR** ;  $\delta$  ppm, 125 MHz, in dimethylsulfoxide-d<sub>6</sub> ; Figure 29  
61.18, 70.25, 73.75, 77.11, 77.41, 102.38, 116.18, 118.40,  
151.28, 153.12
- <sup>13</sup>C NMR** ;  $\delta$  ppm, 125 MHz, in pyridine-d<sub>5</sub>  
62.5, 71.4, 75.1, 78.6, 78.7, 103.6, 116.8, 118.8, 151.9,  
154.1

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