CHAPTER II

ISOLATION AND CHARACTERIZATION OF COMPOUNDS FROM *Andrographis paniculata* AND *Caesalpinia bonduc*

2.1 Experimental Section

2.1.1 Plant material

The whole plant of *Andrographis paniculata* (fha-ta-lai-jone) was collected from Ayutthaya Province, Thailand in July 2005. The plant was identified by comparing with herbarium specimens (BKF no. 12195) in the Royal Forest Department, Ministry of Agriculture and Co-operatives, Bangkok, Thailand.

The seed kernels of *Caesalpinia bonduc* (swat) were collected from Chachoengsao Province, Thailand in November 2005. The plant was identified by comparing with herbarium specimens (BKF no. 55398) in the Royal Forest Department, Ministry of Agriculture and Co-operatives, Bangkok, Thailand.

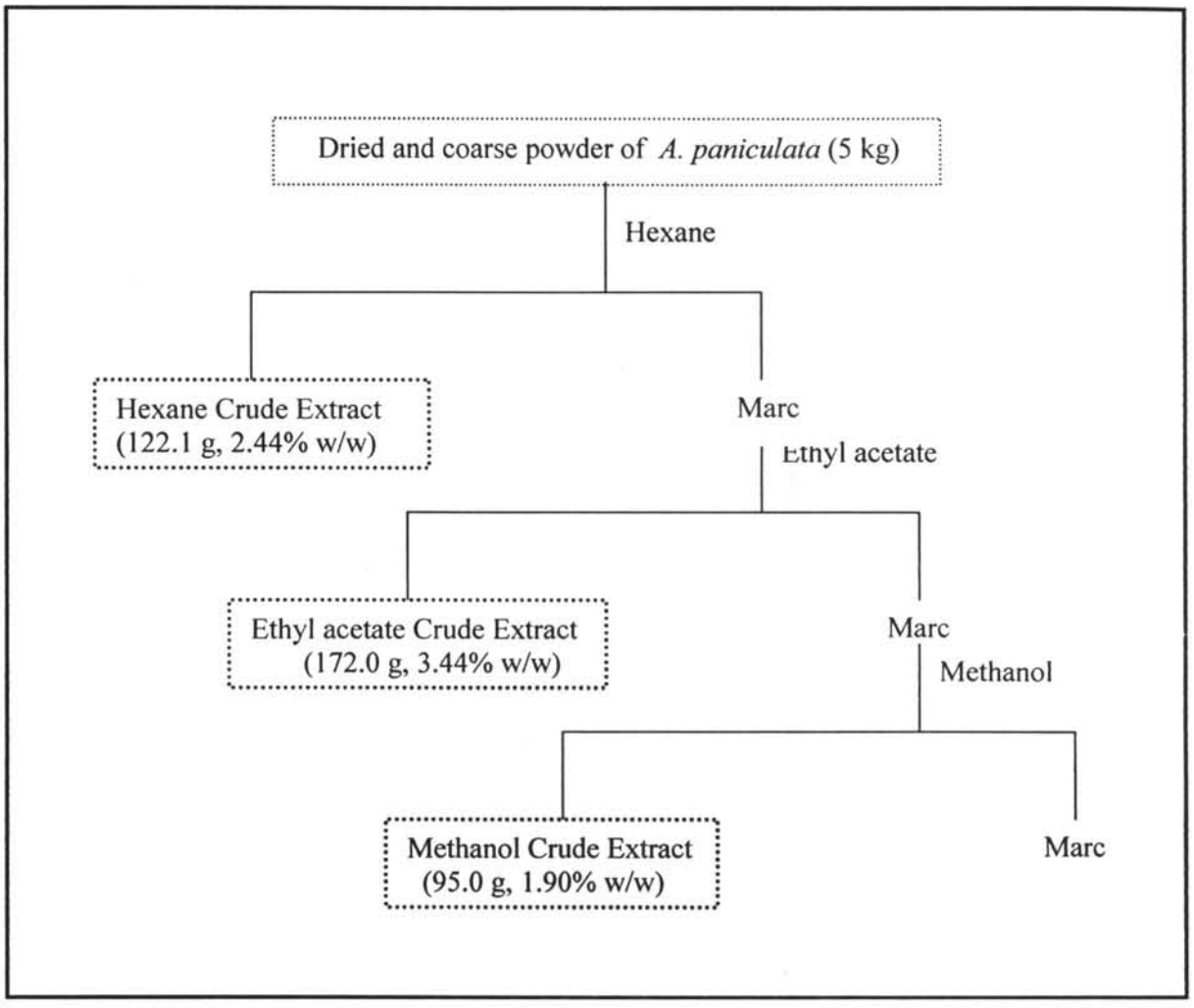
2.1.2 General Experimental Procedure

Melting points (uncorrected) were recorded on Electrothermal 9100 melting apparatus. Optical rotations were measured on Perkin Elmer Model 341 polarimeter. UV spectra were recorded on Cary 50 Probe UV-Visible spectrophotometer. FT-IR spectra were recorded on Perkin Elmer spectrophotometer. NMR spectra were recorded on a Varian Mercury 400 NMR Spectrometer operating 400 MHz for ^{1}H and 100 MHz for ^{13}C. The chemical shifts in δ (ppm) were assigned with reference to the signals from residual proton ($\delta_{7.26}$) and carbon ($\delta_{77.16}$) in deuterated chloroform, proton ($\delta_{3.31}$) and carbon ($\delta_{49.0}$) in deuterated methanol, proton ($\delta_{2.5}$) and carbon ($\delta_{39.52}$) in deuterated dimethyl sulfoxide.

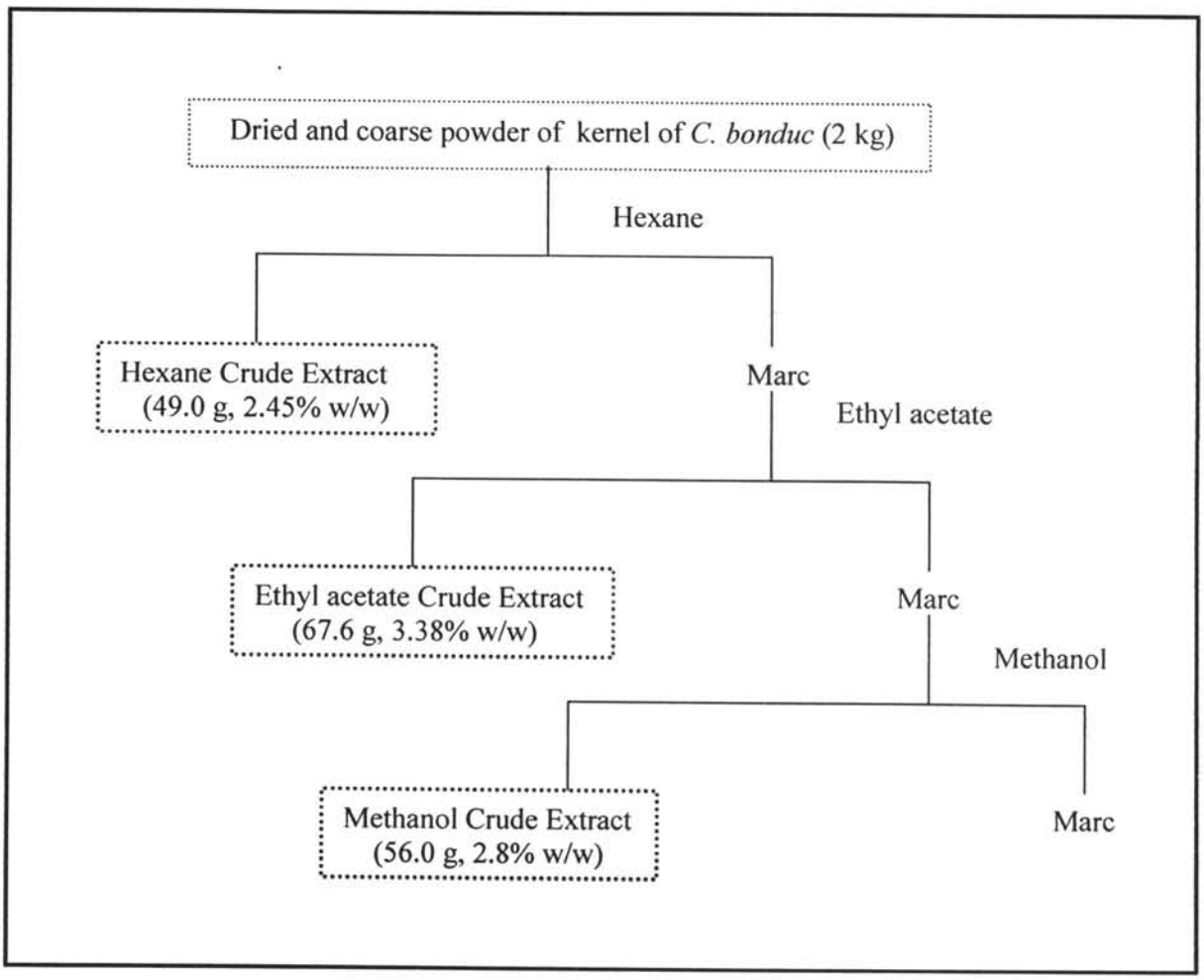
Solvents for extraction, chromatography, and recrystallization were distilled prior to use. Thin-layer chromatography (TLC) was performed on precoated Merck silica gel 60 F_{254} plates (0.25 mm. thick layer) and reverse phase thin-layer chromatography was performed on precoated Merck RP-18 F_{254S} plates. Spots were detected under UV (254 nm) before spraying with phosphomolybdic acid solution in EtOH or *p*-anisaldehyde-acetic solution followed by heating the plate at 150^{O}C. Silica gel 60 Merck No. 7734, No. 9385 and Diaion HP-20 (Wakogel®100 C18) were used for column chromatography.

2.1.3 Extraction

The whole plant of *A. paniculata* and seed kernels of *C. bonduc* were sun dried for a week. Five kilograms of dried whole plant and two kilograms of seed kernels were crush into powder and exhaustively extracted by maceration at room temperature with hexane, ethyl acetate, and methanol thrice for each solvent. Extracts of each solvent were filtrated and evaporated to dryness in vacuum. The extraction of *A. paniculata* afforded 122.1 g of hexane crude extract, 172.0 g of ethyl acetate crude extract, and 95.0 g of methanol crude extract. The extraction of *C. bonduc* gave 49.0 g of hexane crude extract, 67.6 g of ethyl acetate crude extract, and 56.0 g of methanol crude extract, respectively. The procedure of the extraction was summarized in **scheme 2.1** and **2.2**, respectively.



Scheme 2.1 The extraction procedure of the whole plant of *A. paniculata*.



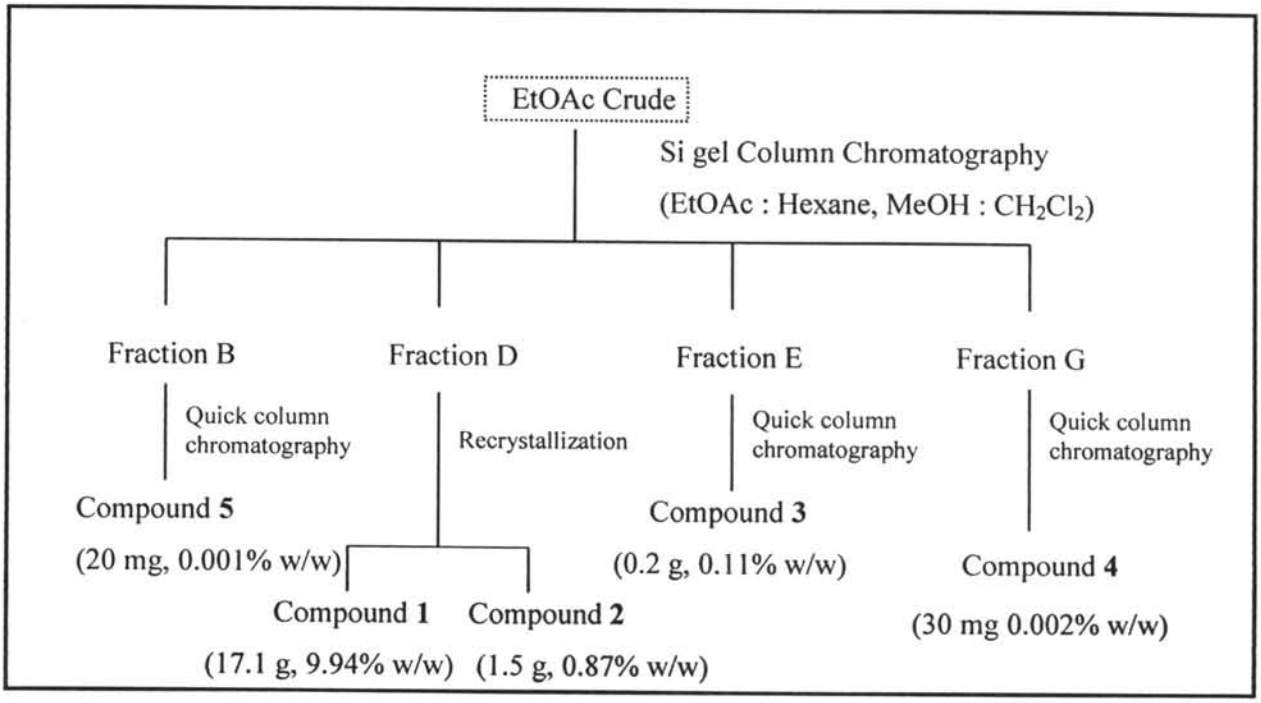
Scheme 2.2 The extraction procedure of the seed kernels of *C. bonduc*.

2.2 Isolation and purification

2.2.1 Isolation and purification of compounds from crude extract of *A. paniculata*

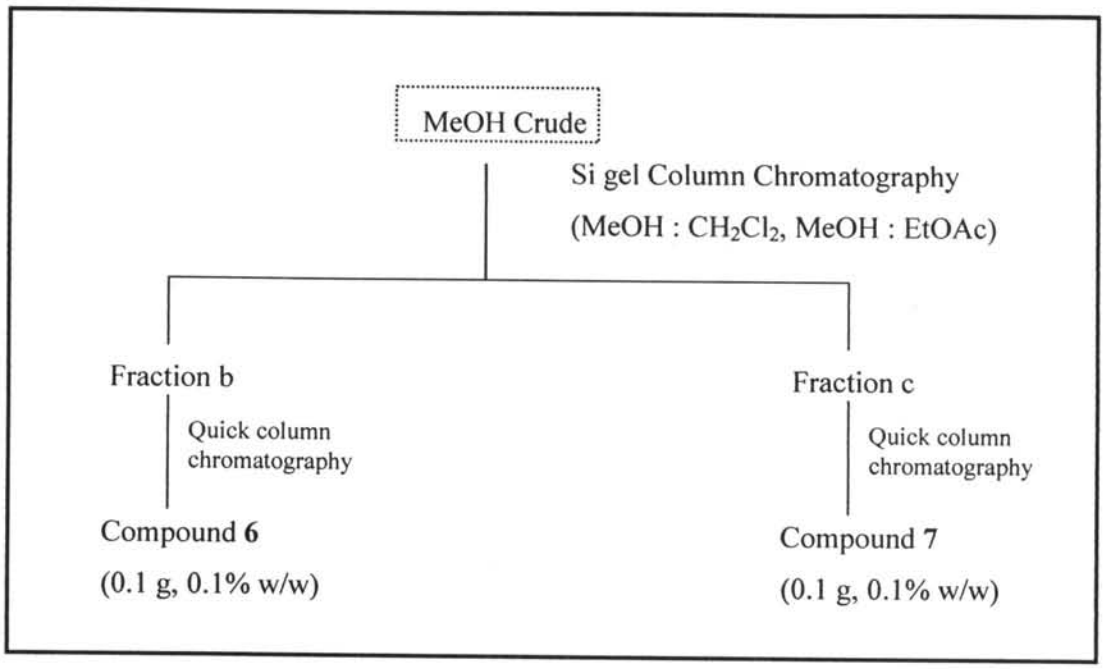
The ethyl acetate crude extract (172.0 g) of *A. paniculata* was chromatographed over a silica gel quick column with a step-wise of EtOAc : hexane and MeOH : CH_2Cl_2. Seven fractions (A-G) were collected according to TLC analysis. Recrystallization of fraction D with MeOH : $CHCl_3$ (1:1) to give Andrographolide (**1,** 17.1 g) and 14-Deoxy-11,12-didehydroandrographolide (**2**, 1.5 g). Fraction B eluted with 30% EtOAc : hexane was rechromatographed on flash column chromatography to furnish 5,2'-Dihydroxy-7,8-dimethoxyflavone (**5**, 20 mg).

Fraction E eluted with 5% MeOH : CH_2Cl_2 and fraction F eluted with 10% MeOH : CH_2Cl_2 were rechromatographed on SiO_2 flash column chromatography to give Neoandrographolide (**3**, 0.2 g), and Andrographiside (**4**, 30 mg), respectively. The isolation and purification procedure were briefly summarized in **Scheme 3.3**



Scheme 2.3 The isolation and purification procedure of Andrographolide (**1**), 14-Deoxy-11,12-didehydroandrographolide (**2**), Neoandrographolide(**3**), Andrographiside (**4**), and 5,2'-Dihydroxy-7,8-dimethoxyflavone (**5**)

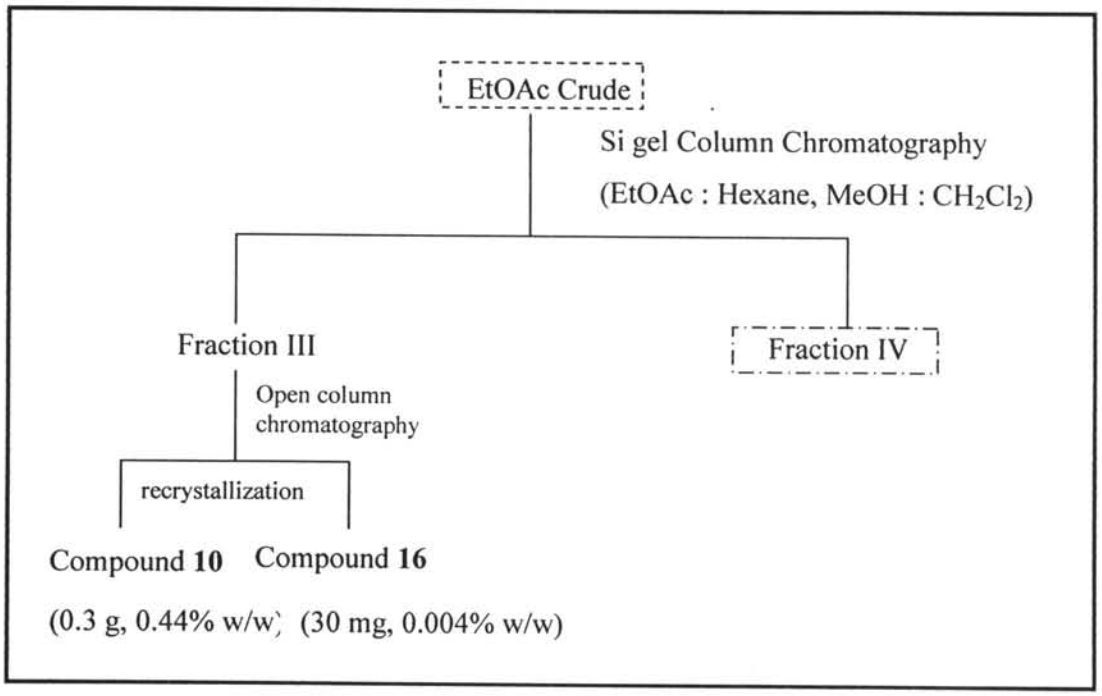
The methanol crude extract (95.0 g) of *A. paniculata* was chromatographed over a silica gel quick column with a step-wise of MeOH : CH_2Cl_2 and MeOH : EtOAc. Three fractions (a-c) were collected according to TLC analysis. Fraction b eluted with 20% MeOH : EtOAc was rechromatographed on flash column chromatography to furnish 8-*O*-Acetylharpagide (**6**, 0.1 g). Fraction c eluted with 25% MeOH : EtOAc was rechromatographed on flash column chromatography to furnish Antirrinoside (**7**, 0.1 g). The isolation and purification procedure were briefly summarized in **scheme 2.4**



Scheme 2.4 The isolation and purification procedure of 8-*O*-Acetylharpagide (**6**), Antirrinoside (**7**).

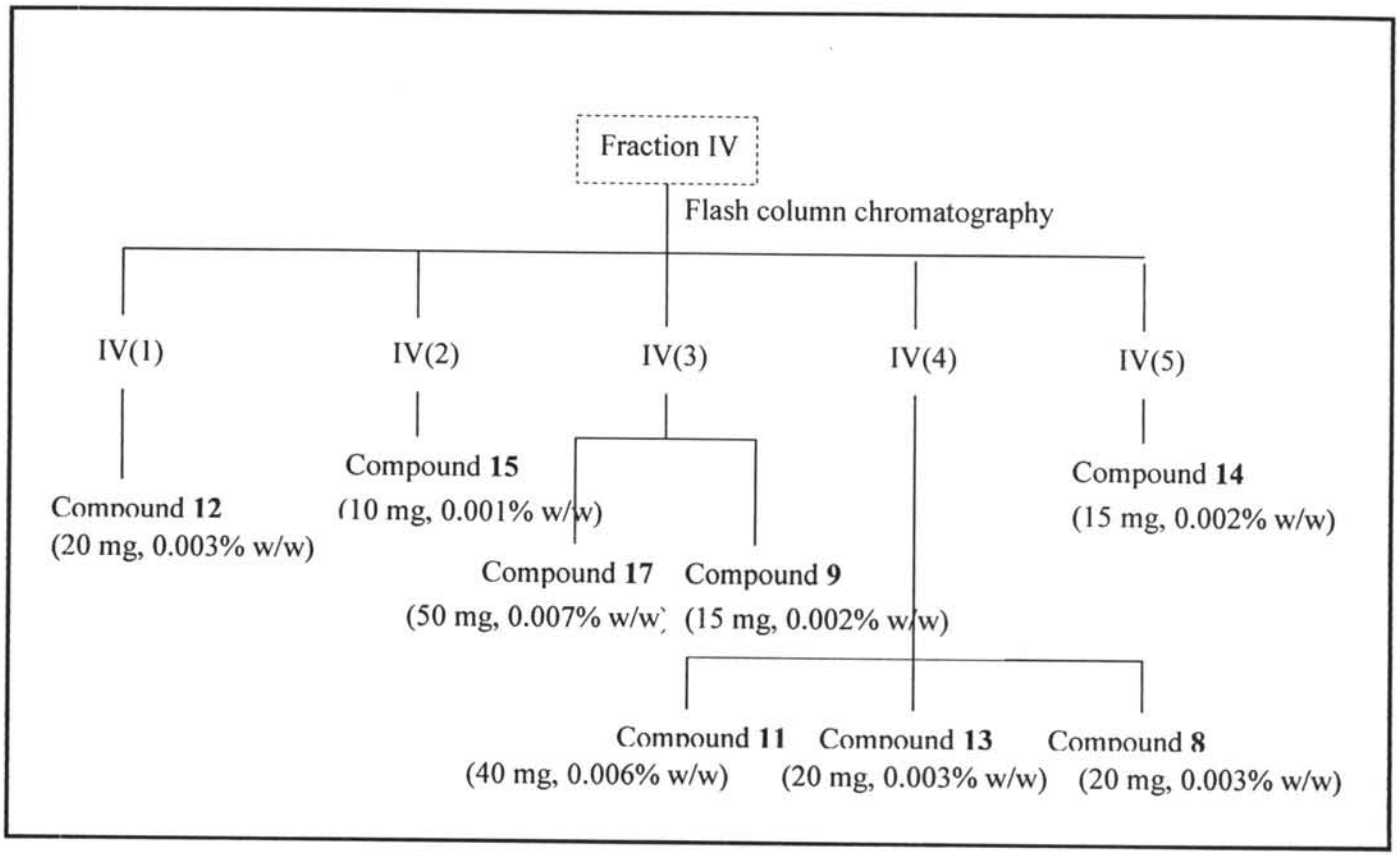
2.2.2 Isolation and purification of compounds from crude extract of *C. bonduc*

The ethyl acetate crude extract (67.6 g) of *C. bonduc.* was chromatographed over a silica gel quick column with a step-wise of EtOAc : hexane and MeOH : CH_2Cl_2. Six fractions (I-VI) were collected according to TLC analysis. Fraction III eluted with 30% EtOAc : Hexane was recrystallized with MeOH to give 14(17)-Dehydrocaesalpin F (**10**, 0.3 g) and the mother liquid was then evaporated and rechromatographed on open column chromatography to furnish 2-Acetoxycaesal dekarin e (**16**, 30 mg). The isolation and purification procedure were briefly summarized in **scheme 2.5**



Scheme 2.5 The isolation and purification procedure of 14(17)-Dehydro caesalpin F (**10**) and 2-Acetoxycaesaldekarin e (**16**).

Fraction IV eluted with 50% EtOAc : hexane and 100% EtOAc was rechromatographed on flash column chromatography to afford five subfractions (IV(1) – IV(5)). Fraction IV(1) eluted with 40% MeOH : DI water was rechromatographed on Wakogel®100C1δ column chromatography to furnish Bonducellpins C (**12**, 20 mg). Fraction IV(2) eluted with 25% EtOAc : hexane was rechromatographed on open column chromatography to give Caesalpinin I (**15**, 10 mg). Fraction IV(3) eluted with 2.5% MeOH : $CHCl_3$ was rechromatographed on open column chromatography to furnish Caesalpinin Q (**17**, 50 mg) and Caesalpinin P (**9**, 15 mg). Fraction IV(4) eluted with 15% EtOAc : benzene was rechromatographed on open column chromatography to afford ε-Caesalpin (**11**, 40 mg), Caesalpinin K (**13**, 20 mg) and Caesalpinin C (**8**, 20 mg). Fraction IV(5) eluted with 20% EtOAc : benzene was rechromatographed on open column chromatography to give Caesalmin B (**14**, 15 mg). The isolation and purification procedure were briefly summarized in **scheme 2.6**.



Scheme 2.6 The isolation and purification procedure of Bonducellpins C (**12**), Caesalpinin I (**15**), Caesalpinin Q (**17**), Caesalpinin P (**9**), ε-Caesalpin(**11**), Caesalpinin K (**13**), Caesalpinin C (**8**), and Caesalmin B (**14**).

2.3 Structural Elucidation

2.3.1 Compounds isolated from *A. paniculata*

Compound **1** was obtained as a colorless crystal (m.p. 230-232 °C) and its molecular formula was established to be $C_{20}H_{30}O_5$ by the NMR spectroscopic data.[19] Based on the NMR spectral data and literature (**Table 2.1**) data comparison, compound **1** was identified to be Andrographolide [19], which is a major compound in this plant.

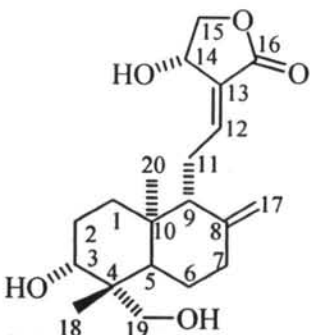


Figure 2.1 Structure of Andrographolide (**1**)

Table 2.1 The ^{1}H and ^{13}C NMR spectral data of Andrographolide and compound **1**.

Position	Andrographolide		Compound 1	
	δ_C	δ_H (multiplicity *J* in Hz)	δ_C	δ_H (multiplicity *J* in Hz)
1	38.1	1.80 m	37.5	1.80 m
		1.28 m		1.29 m
2	29.0	1.78 m	27.6	1.77 m
3	80.9	3.38 m	79.5	3.38 m
4	43.7	-	46.9	-
5	56.3	1.32 m	54.9	1.33 m
6	25.2	1.28 m	23.8	1.29 m
		1.90 m		1.90 m
7	39.0	2.04 m	38.5	2.04 m
		2.42 m		2.42 m
8	148.8	-	147.3	-
9	57.4	1.91 dd (9.4,5.0)	55.9	1.90 dd (9.4,5.0)
10	40.0	-	42.2	-
11	25.7	2.58 m	24.2	2.59 m
12	149.3	6.83 dd (6.7,5.4)	147.9	6.84 t (1.2)
13	129.8	-	128.4	-
14	66.7	5.00 d (6.0)	65.2	5.00 d (5.6)
15	76.1	4.46 dd (6.7,5.4)	74.7	4.46 dd (10.4,6.0)
16	172.6	-	171.2	-
17	109.2	4.87 s	107.8	4.88 s
18	23.4	1.28 s	22.0	1.21 s
19	65.0	4.12 d (11.5)	65.0	4.22 d (10.4,2.0)
		3.35 d (11.5)		3.35 d (11.5)
20	15.5	0.74 s	14.1	0.75 s

Compound **2** was isolated as a colorless crystal (m.p. 204 - 205 °C) and its molecular formula was established to be $C_{20}H_{28}O_4$ by the NMR spectroscopic data.[19] Comparison of spectroscopic data to these published in the literature (**Table 2.2**) indicated that compound **2** was 14-Deoxy-11,12-didehydroandrographolide.[19]

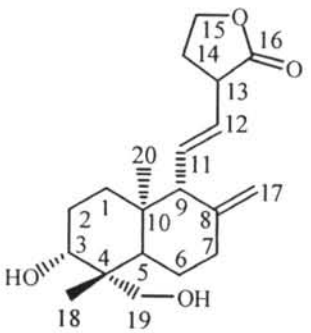


Figure 2.2 Structure of 14-Deoxy-11,12-didehydroandrographolide (**2**)

Table 2.2 The ^{1}H and ^{13}C NMR spectral data of 14-Deoxy-11,12-didehydro andrographolide and compound **2**

Position	14-Deoxy-11,12-didehydro andrographolide		Compound 2	
	δ_C	δ_H (multiplicity *J* in Hz)	δ_C	δ_H (multiplicity *J* in Hz)
1	39.5	1.83 m	38.5	1.83 m
		1.34 m		1.33 m
2	28.9	1.75 m	27.6	1.75 m
3	81.2	3.34 m	79.6	3.35 m
4	43.8	-	42.2	-
5	55.8	1.35 m	55.9	1.35 m
6	24.4	1.81 m	24.2	1.81 m
7	37.8	2.03 m	37.9	2.03 m
		2.36 m		2.36 m
8	150.1	-	147.9	-
9	62.8	1.84 m	61.4	1.86 m
10	39.6	-	38.7	-
11	136.5	6.85 dd (15.6,10.1)	133.3	6.85 dd (15.8,10.1)
12	122.5	6.16 d (15.6)	121.1	6.15 d (15.8)
13	129.6	-	128.4	-
14	146.6	7.44 brt	146.2	7.43 brt
15	71.6	4.35 m	70.7	4.35 m
16	174.8	-	171.2	-
17	109.2	4.87 s	107.8	4.88 s
		5.00 d (5.6)		4.81 d (1.8)
18	23.3	1.22 s	23.0	1.21 s
19	65.0	4.13 d (11.1)	65.2	4.12 m
		3.38 d (11.1)		3.38 m
20	16.3	0.84 s	14.5	0.84 s

Compound **3** was obtained as a colorless amorphous solid (m.p. 201-203 °C) and its molecular formula, $C_{26}H_{41}O_8$, was determined by the NMR spectroscopic data.[19,34] Comparison of spectroscopic data to these published in the literature (**Table 2.3**) indicated that compound **3** was Neoandrographolide.[19]

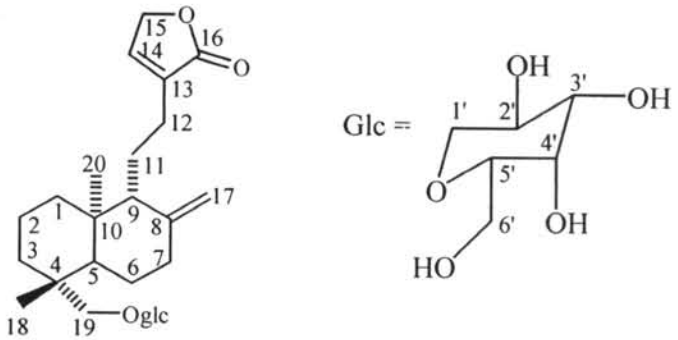


Figure 2.3 Structure of Neoandrographolide (**3**)

Table 2.3 The ^1H and ^{13}C NMR spectral data of Neoandrographolide and compound **3**

Position	Neoandrographolide δ_C	Neoandrographolide δ_H (multiplicity *J* in Hz)	Compound 3 δ_C	Compound 3 δ_H (multiplicity *J* in Hz)	Position	Neoandrographolide δ_C	Neoandrographolide δ_H (multiplicity *J* in Hz)	Compound 3 δ_C	Compound 3 δ_H (multiplicity *J* in Hz)
1	39.0	1.08 m	38.7	1.08 dt (3.6,13.2)	15	70.6	4.72 d (1.5)	70.6	4.81 s
		1.34 m		1.33 m	16	174.6	-	175.6	-
2	19.3	1.75 m	18.6	1.75 m	17	106.9	4.89 s	105.8	4.86 brs
3	36.4	0.90 m	35.7	0.94 dt (3.6,13.6)			4.7 s		4.63 brs
		2.13 brt (13)		1.95 brd (13.2)	18	28.1	1.17 s	26.9	1.03 s
4	39.8	-	37.9	-	19	72.5	4.32 d (9.5)	71.9	4.09 d (9.2)
5	56.2	1.60 m	56.4	1.67 m			3.48 d (9.5)		3.20 brd (9.6)
6	24.7	1.81 m	24.1	1.81 m	20	16.6	0.63 s	14.4	0.70 s
7	38.7	2.03 m	38.2	1.98 m	1'	105.5	4.82 d (7.5)	103.6	4.16 d (8)
		2.36 m		2.41 m	2'	75.3	4.03 d (7.5)	73.8	3.15 dd (8,8.8)
8	148.1	-	147.7	-	3'	78.7	4.20	76.8	3.32 dd (7.6)
9	56.6	1.60 m	56.2	1.26 dd (1.8,13)	4'	71.7	4.20	70.2	3.28 dd (8.4)
10	38.6	-	39.0	-	5'	78.4	3.95 m	76.3	3.23 m
11	22.0	1.60 m	21.5	1.61 m	6'	62.8	4.54 dd (5.5,11.5)	61.3	3.84 dd (2,12)
		1.70 m		1.77 m			4.38 dd (5.5,11.5)		3.67 dd (5.2,12)
12	24.9	2.20 m	24.0	2.09 dt					
		2.50 m		2.38 m					
13	134.1	-	133.3	-					
14	145.3	7.15 brt	146.2	7.33 s					

Compound **4** was obtained as colorless plates (m.p. 201-203 °C) and the molecular formula was determined to be $C_{26}H_{41}O_{10}$ by the NMR spectroscopic data[19]. Comparison of spectroscopic data to these published in the literature (**Table 2.4**) indicated that compound **4** was Andrographiside[19].

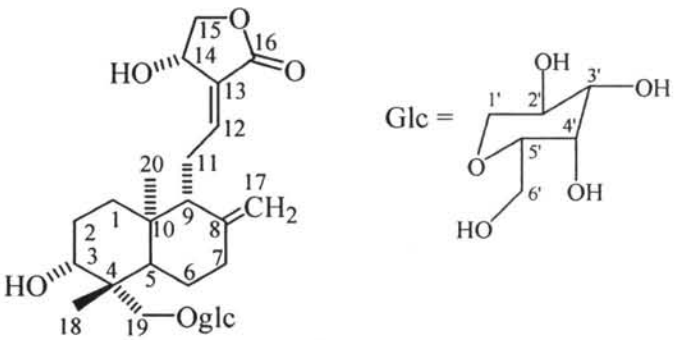


Figure 2.4 Structure of Andrographiside (**4**)

Table 2.4 The ^{1}H and ^{13}C NMR spectral data of Androgaphiside and compound **4**

	Andrographiside		**Compound 4**			**Andrographiside**		**Compound 4**	
Position	δ_C	δ_H (multiplicity *J* in Hz)	δ_C	δ_H (multiplicity *J* in Hz)	**Position**	δ_C	δ_H (multiplicity *J* in Hz)	δ_C	δ_H (multiplicity *J* in Hz)
1	37.9	1.8 m	37.5	1.84 m	15	75.2	4.46 dd	74.7	4.42 dd
		1.28 m		1.21 m			(10,2.6)		(10,2.6)
2	29.2	1.78 m	27.6	1.72 m			4.58 dd		4.44 dd
3	79.1	3.52 dd	79.5	3.45 dd			(10,5.9)		(10,5.9)
		(4,11.6)		(4,11.6)	16	170.6	-	171.2	-
4	43.3	-	46.9	-	17	108.5	4.81 s	107.8	4.79 brs
5	55.7	1.32 m	54.9	1.57 m			4.85 s		4.80 brs
6	25.0	1.28 m	23.8	1.21 m	18	24.4	1.45 s	22.0	1.57 s
7	38.4	2.04 m	38.5	1.88 m	19	72.0	3.85 d	65.0	3.9 d
		2.42 m		2.32 m			(10.5)		(10.5)
8	148.2	-	147.3	-			4.61 d (10.5)		4.61 d (10.5)
9	56.6	1.91 dd	55.9	1.86 dd	20	14.8	0.84 s	14.4	0.73 s
		(9.4,5.0)		(9.4,5.0)	1'	105.4	4.79 d (7.8)	103.6	4.83 d (7.8)
10	39.5	-	42.2	-	2'	74.9	3.95 t (7.8)	74.8	3.92 t (7.8)
11	25.1	2.73 t (7)	24.2	2.9. t (7)	3'	78.7	4.1	77.9	3.94
12	146.9	7.17 td	147.9	6.63 td	4'	71.8	4.1	71.2	3.94
		(6.7,1.6)		(6.7,1.6)	5'	78.4	3.88 m	78.3	3.67 m
13	130.1	-	128.4	-	6'	62.8	4.29 dd	61.3	4.02 dd
14	66.0	5.32 brs	65.2	4.91 brs			(5.4,11.6)		(5.4,11.6)
							4.46 dd		4.04 dd
							(2.4,11.6)		(2.4,11.6)

Compound **5** was isolated as yellow needles (m.p. 263-265 °C). It showed the characteristic ^{1}H NMR signals of flavones with 5,7,8-trioxygenation.[48] The structure of compound **5** was established by analysis of ^{1}H-^{1}H COSY, HSQC and HMBC spectra as shown in **Figure 2.5**. Base on the literature search, compound **5** was 5,2'-Dihydroxy-7,8-dimethoxyflavone, which was previously isolated from tissue cultures of *A. paniculata*[22] and flavonoid glycoside from *A. alata*.[35]

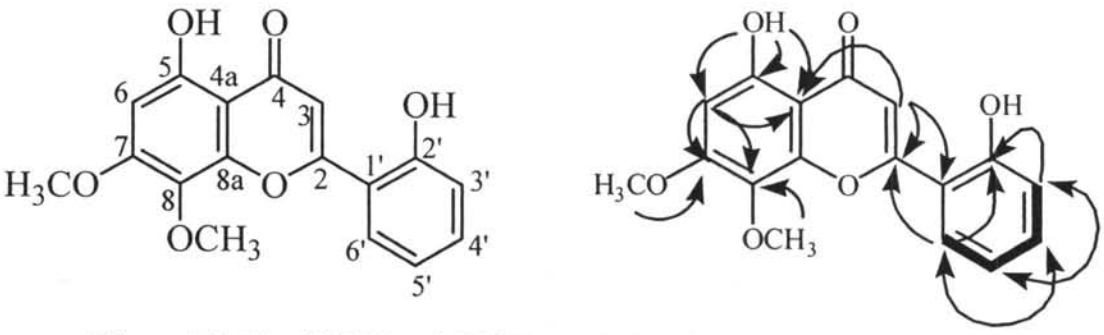


Figure 2.5 Key HMBC and COSY correlations for 5,2'-Dihydroxy-7,8-dimethoxyflavone (**5**)

Table 2.5 The ^{1}H and ^{13}C NMR spectral data of compound **5**

Position	Compound 5	
	δ_C	δ_H (multiplicity *J* in Hz)
2	162.0	-
3	109.2	7.13 s
4	182.8	-
4a	104.3	-
5	157.1	-
6	96.3	6.60 s
7	158.9	-
8	128.9	7.88 dd (8.0,1.2)
8a	149.4	-
1'	117.6	7.07 d (8.4)
2'	157.6	-
3'	117.6	7.07 d (8.4)
4'	133.5	7.4 dt (8.0,1.2)
5'	119.9	7.04 d (7.2)
6'	128.7	-
7-OMe	56.5	3.92 s
8-OMe	61.5	3.82 s
6-OH	-	12.72

Compound **6** was obtain as colorless plates (m.p. 227-229 °C) and its molecular formula was established to be $C_{17}H_{26}O_{11}$ by the NMR spectroscopic data.[36] Comparison of spectroscopic data to these published in the literature (**Table 2.6**) indicated that compound **6** was 8-*O*-Acetylharpagide, previously isolated from *Ajuga reptans*.[36,37] 8-*O*-Acetylharpagide (**6**) was isolated from *A. paniculata* for the first time in this study.

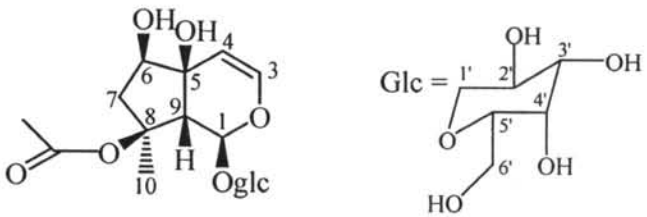


Figure 2.6 Structure of 8-*O*-Acetylharpagide (**6**)

Table 2.6 The ^{1}H and ^{13}C NMR spectral data of 8-*O*-Acetylharpagide and compound **6**

Position	8-*O*-acetylharpagide		Compound 6	
	δ_C	δ_H (multiplicity *J* in Hz)	δ_C	δ_H (multiplicity *J* in Hz)
1	94.5	6.06 s	92.4	5.97 s
3	143.8	6.38 d (6.4)	141.9	6.44 d (6.4)
4	106.8	4.90 dd (6.4,1.6)	102.0	5.14 dd (6.4,1.2)
5	73.3	-	71.8	-
6	77.6	3.71 dd (4.4,1.2)	74.8	4.21 dd (12.0,6.4)
7	45.9	1.93 dd (15.1,4.4)	44.0	1.55 dd (13.2,6.8)
		2.14 dd (15.1,4.4)		2.17 dd (13.6,6.4)
8	88.6	-	83.5	-
9	55.3	2.84 brs	54.8	2.65 s
10	22.4	1.44 s	20.5	1.47 s
1'	99.8	4.58 d (7.9)	98.2	4.57 d (8.0)
2'	74.4	3.19 dd (9.2,7.9)	73.1	3.17 t (8.4)
3'	77.5	3.38 dd (9.2,7.9)	76.2	3.36 m
4'	71.6	3.30	70.2	3.34
5'	78.1	3.30	76.7	3.29
6'	62.7	3.88 dd (12.1,1.6)	61.3	3.88 d (12.0)
		3.68 dd (12.1,5.5)		3.68 dd (11.2,4.0)
8-OCO<u>CH</u>$_3$	22.2	2.01 s	20.7	2.01 s
8-O<u>C</u>OCH$_3$	173.3	-	171.8	-

Compound **7** was obtained as a brown oil. The ^{1}H and ^{13}C NMR spectra (**Table 2.7**) were closely related to those of 8-*O*-Acetylharpagide (**6**), except for the presence of one more oxymethine (δ_H 3.30 and δ_C 64.4) accompanied by the absence of the acetyl group. From the significant upfield shift of oxymethine resonances at δ_C 64.4 and 65.4, the epoxide ring was assigned to be located at C-7 and C-8. The structure of compound **7** was established by analysis of ^{1}H-^{1}H COSY, HSQC and HMBC spectra as shown in **Figure 2.7**. Base on the literature search, compound **7** was Antirrinoside, which was previously isolated from *Kickxia abhaica*.[49] Antirrinoside (**7**) was also isolated from *A. paniculata* for the first time in this study.

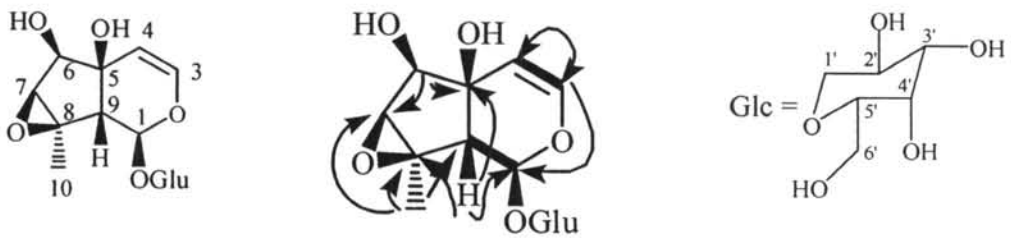


Figure 2.7 Key HMBC and COSY correlations for Antirrinoside (**7**)

Table 2.7 The ^{1}H and ^{13}C NMR spectral data of compound **7**

Position	Compound 7	
	δ_C	δ_H (multiplicity *J* in Hz)
1	94.1	5.39 d (8.4)
3	142.7	6.45 d (6.0)
4	103.0	4.82 d (6.0)
5	78.7	-
6	77.0	3.82 brs
7	64.4	3.30 m
8	65.4	-
9	51.6	2.25 d (8.4)
10	16.2	1.53 s
1'	98.2	4.70 d (8.0)
2'	73.4	3.23 m
3'	76.4	3.38 m
4'	70.3	3.27 m
5'	77.1	3.29 m
6'	61.6	3.64 dd (12.0,6.0)
		3.87 dd (12.0,1.6)

2.3.2 Compounds isolated from *C. bonduc*

Compound **8** was isolated as pale yellow oil. The ^{1}H NMR spectrum (**Table 2.8**) displayed signals corresponding to three tertiary methyls (δ_H 1.16, 1.15, and 1.11), two oxygen-substituted methines (δ_H 4.95 and 4.88), two aliphatic methines (δ_H 2.87 and 2.29), two protons of an exomethylene group (δ_H 5.12 and 4.93) together with two protons of a 1,2-disubstituted furan ring (δ_H 7.27 and 6.45) and two acetyl methyls (δ_H 2.05 and 2.03). Analysis of ^{13}C NMR (**Table 2.8**) and HSQC spectrum revealed the presence of 20 nonequivalent carbons, including six olefinic carbons (δ_C 151.5, 142.2, 141.6, 119.0, 106.3, and 104.4) and three oxygen-substituted carbons (δ_C 76.9, 76.7, and 73.8) together with two ester carbonyl groups (δ_C 169.5 and 169.3). These data suggested that Compound **8** was a cassane diterpenoid. The partial structures elucidated from the ^{1}H-^{1}H COSY study are indicated by bold lines and the crucial ^{1}H-^{13}C correlations (HMBC) shown by arrow in **Figure 2.8**.

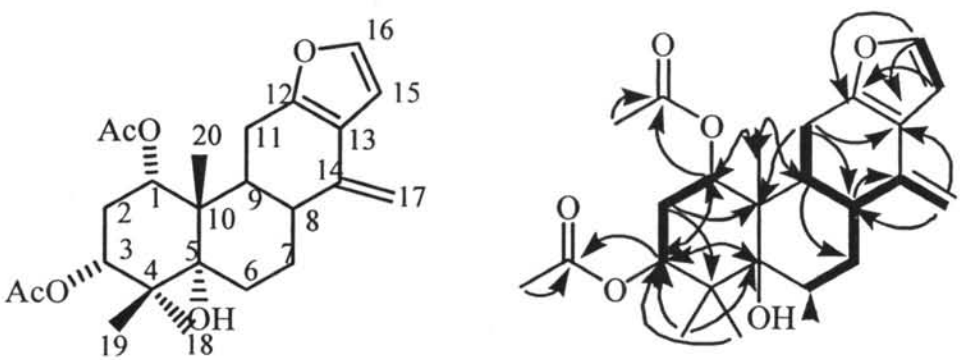


Figure 2.8 Key HMBC and COSY correlations for compound **8**.

Based on the NMR spectral data and literature data comparison (**Table 2.8**), compound **8** was identified as caesalpinin C, previously isolated from *Caesalpinia crista* of Indonesia.[41]

Table 2.8 The ^{1}H and ^{13}C NMR spectral data of Caesalpinin C and compound **8**

Position	Caeselpinin C		Compound 8	
	δ_C	δ_H (multiplicity *J* in Hz)	δ_C	δ_H (multiplicity *J* in Hz)
1	73.8	4.88 t (3.1)	73.8	4.88 t (3.1)
2	26.5	2.37 m	26.5	2.32 m
		2.15 m		2.14 m
3	76.9	4.95 t (3.1)	76.9	4.95 t (3.1)
4	41.6	-	41.6	-
5	76.7	-	76.7	-
6	23.4	1.93 m	26.4	1.79 m
		1.78 m		-
7	22.3	1.21 m	23.4	1.93 m
		2.06 m		2.04 m
8	35.1	2.36 m	35.0	2.29 m
9	38.7	2.87 td (11.2,5.6)	38.7	2.87 td (11.2,5.6)
10	43.8	-	43.8	-
11	26.5	2.54 dd (16.1,11.2)	22.3	2.54 dd (16.1,11.2)
		2.30 dd (16.1,5.6)		2.30 dd (16.1,5.6)
12	151.3	-	151.3	-
13	119.1	-	119.0	-
14	142.2	-	142.2	-
15	106.3	6.45 d (2.5)	106.3	6.45 d (2.5)
16	141.5	7.23 d (2.5)	141.6	7.27 d (2.5)
17	104.3	4.92 d (2.7)	104.4	4.93 d (2.7)
		5.12 d (2.7)		5.12 d (2.7)
18	23.1	1.11 s	23.1	1.11 s
19	25.4	1.15 s	25.4	1.15 s
20	18.0	1.16 s	18.0	1.16 s
1-OCO$\underline{CH_3}$	21.4	2.04 s	21.4	2.05 s
1-O$\underline{C}$OCH$_3$	169.4	-	169.5	-
3-OCO$\underline{CH_3}$	21.1	2.03 s	21.2	2.03 s
3-O$\underline{C}$OCH$_3$	169.3	-	169.3	-
5-OH		3.27 brs		3.27 brs

Compound **9** was also isolated as pale yellow oil. The ^{1}H and ^{13}C NMR spectra (**Table 2.9**) were similar to those of Caesalpinin C (**8**), except for the position of one of the acetyl substituents. The location of the acetyl group was determined to be C-2 instead of C-3 in compound **8** by the analysis of the COSY, HSQC, and HMBC spectra (**Figure 2.9**). Comparison of spectroscopic data to those published in the literature (**Table 2.9**) confirmed that compound **9** was Caesalpinin P, previously isolated from *Caesalpinia crista* of Indonesia.[41]

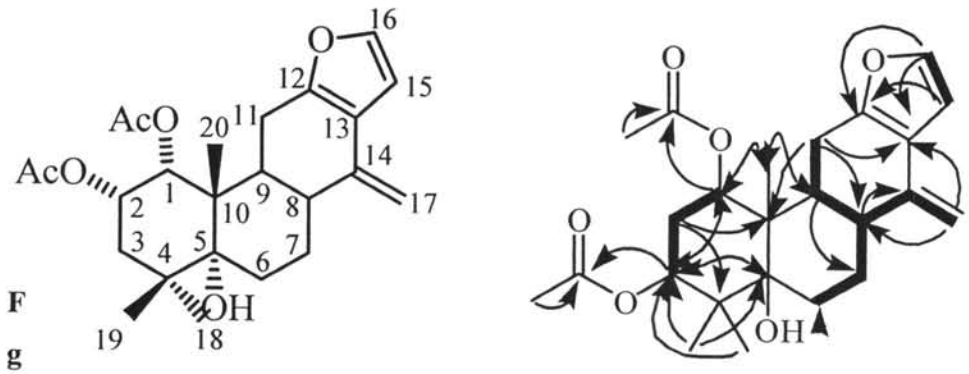


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Figure 2.9 Key HMBC and COSY correlations for compound **9**

Table 2.9 The ^{1}H and ^{13}C NMR spectral data of Caesalpinin P and compound **9**.

Position	Caesalpinin P		Compound 9	
	δ_C	δ_H (multiplicity *J* in Hz)	δ_C	δ_H (multiplicity *J* in Hz)
1	74.8	5.26 brs	74.8	5.26 brs
2	67.5	5.32 m	67.5	5.32 m
3	35.8	1.39 m	35.7	1.38 m
		2.03 m		2.00 m
4	40.2	-	40.2	-
5	77.2	-	76.6	-
6	25.6	1.77 m	25.6	1.76 m
		1.74 m		
7	23.6	1.70 m	23.5	1.93 m
		-		2.04 m
8	35	1.98 m	34.9	2.29 m
9	39.2	2.64 dd (11.7,3.1)	39.2	2.56 dd (11.7,3.1)
10	45.1	-	45.0	-
11	22.5	2.49 dd (16.5,11.7)	22.5	2.53 dd (1.65,11.7)
		2.39 dd (16.5,3.1)		2.40 dd (16.5,3.1)
12	151.3	-	151.3	-
13	119.0	-	118.9	-
14	142.5	-	142.1	-
15	106.2	6.43 d (2.0)	106.2	6.44 d (2.0)
16	141.7	7.22 d (2.0)	141.6	7.22 s
17	104.4	5.11 s	104.4	5.11 s
		4.91 s		4.92 s
18	28.2	1.11 s	25.9	1.20 s
19	26.0	1.20 s	28.2	1.10 s
20	17.5	1.26 s	17.4	1.26 s
1-OCO$\underline{CH_3}$	21.6	2.11 s	21.2	2.12 s
1-O$\underline{C}$OCH$_3$	169.2	-	169.2	-
2-OCO$\underline{CH_3}$	21.0	1.97 s	21.0	1.98 s
2-O$\underline{C}$OCH$_3$	170.4	-	170.4	-

Compound **10** was isolated as a colorless amorphous solid. The ^{1}H and ^{13}C NMR spectra (**Table 2.10**) were also similar to those of Caesalpinin C (**8**) and Caesalpinin P (**9**), but showed the presence of one more acetyl group (δ_C 169.4 and δ_H 2.11) and one more oxymethine (δ_C 77.1 and δ_H 5.16) accompanied by the disappearance of signal due to one of three methylene in compound **8** and compound **9**. The *O*-acetyl groups were attached to C-1, C-2, and C-3 by analysis of the COSY and HMBC spectra (**Figure 2.10**). Comparison of the ^{1}H and ^{13}C NMR spectral data to those published in the literature (**Table 2.10**) confirmed that compound **10** was 14(17)-Dehydrocaesal F, previously isolated from *Caesalpinia crista* of Indonesia[41] and obtained from semi-synthesis of caesalpin F.[44]

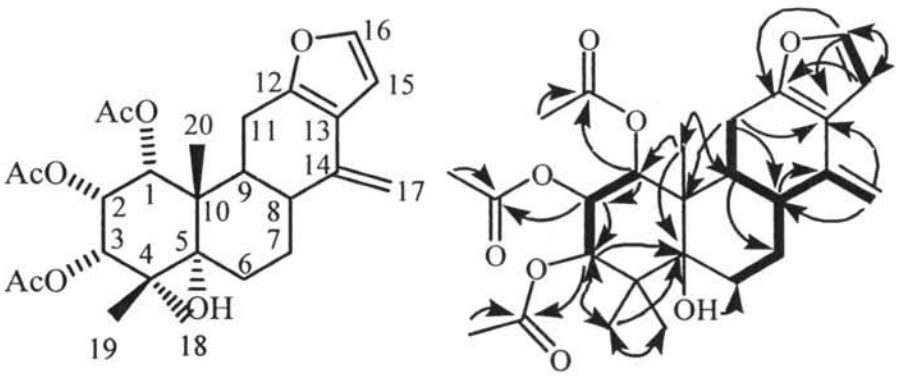

Figure 2.10 Key HMBC and COSY correlations for compound **10**

Table 2.10 The ^{1}H and ^{13}C NMR spectral data of compound **10**

Position	Compound 10		Position	Compound 10	
	δ_C	δ_H (multiplicity *J* in Hz)		δ_C	δ_H (multiplicity *J* in Hz)
1	73.8	5.26 brs	14	142.4	-
2	65.9	5.51 m	15	106.2	6.43
3	77.1	5.16 m	16	141.6	7.26 s
4	43.0	-	17	104.4	4.91 s
5		-			5.11 s
6	76.5	-	18	23.1	1.13 s
7	23.0	1.90 m	19	25.4	1.26 s
		2.00 m	20	18.1	1.28 s
8	34.8	2.3 m	1-OCO$\underline{C}H_3$	21.1	2.07
9	39.0	2.78	1-O$\underline{C}OCH_3$	169.3	-
10	45.3	-	2-OCO$\underline{C}H_3$	20.6	1.97
11	22.4	2.35	2-O$\underline{C}OCH_3$	169.8	-
		2.54	3-OCO$\underline{C}H_3$	20.8	2.11
12	151.2	-	3-O$\underline{C}OCH_3$	169.4	-
13	118.9	-			

Compound **11** was isolated as a colorless oil. The ^{1}H and ^{13}C NMR spectra (**Table 2.11**) were also closely resembled to those of compound **9**, except for the replacement of the exomethylene signal by that of tertiary methyl (δ_C 26.3 and δ_H 1.47) and its ^{13}C NMR spectrum indicated the presence of oxymethine carbon (δ_C 68.8) with the disappearance of one sp^2 carbon assigned for C-14 in compound **9**, indicating the presence of a hydroxyl group at C-14. Analysis of the ^{1}H-^{1}H COSY and HMBC spectra (**Figure 2.11**) revealed that compound **11** was ε-Caesalpin, previously isolated from *Caesalpinia minax*.[42]

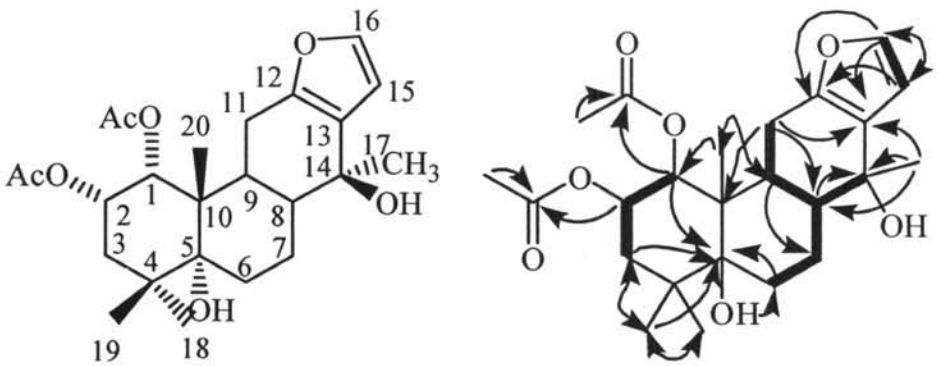

Figure 2.11 Key HMBC and COSY correlations for compound **11**

Table 2.11 The ^{1}H and ^{13}C NMR spectral data of compound **11**

Position	Compound 11		Position	Compound 11	
	δ_C	δ_H (multiplicity *J* in Hz)		δ_C	δ_H (multiplicity *J* in Hz)
1	74.9	5.27 brs	13	123	-
2	67.6	5.30 m	14	68.8	-
3	35.7	1.39 m	15	107.4	6.40 s
		2.02 m	16	141.3	7.22 s
4	40.2	-	17	26.3	1.47 s
5	76.3	-	18	28.2	1.13 s
6	25.3	1.72 m	19	26.1	1.20 s
7	18.9	1.71 m	20	17.7	1.23 s
8	41.4	1.64 m	1-OCO$\underline{C}H_3$	21.3	2.15 s
9	34.1	2.70 s	1-O$\underline{C}OCH_3$	169.4	-
10	44.8	-	2-OCO$\underline{C}H_3$	21	1.99 s
11	22.4	2.40 s	2-O$\underline{C}OCH_3$	170.4	-
12	150.3	-			

Compound **12** was obtained as a colorless amorphous solid. The ^{1}H NMR spectrum (**Table 2.12**) showed characteristic signals of two protons of a 1,2-disubstituted furan ring (δ_H 7.24 and 6.17), two oxygen-substituted methines (δ_H 4.91 and 4.01), a methoxy carbonyl (δ_H 3.75), and an acetyl methyl (δ_H 2.05). The acetyl group was located at the C-1 (δ_H 4.91) as showed HMBC cross peaks to C-2, C-3, C-5, C-10, and C-20 (**Figure 2.12**). A signal at δ_H 3.48 was assigned to H-14 due to its HMBC correlations to the methoxy carbonyl at (δ_C 176.1). On the other hands, a signal at δ_H 3.48 was determined to be H-7 by the analysis of ^{1}H-^{1}H COSY and HMBC correlation (**Figure 2.12**). Based on the NMR spectral data and literature data comparison (**Table 2.12**), compound **12** was identified as Bonducellpin C, previously isolated from *Caesalpinia boduc*.[45]

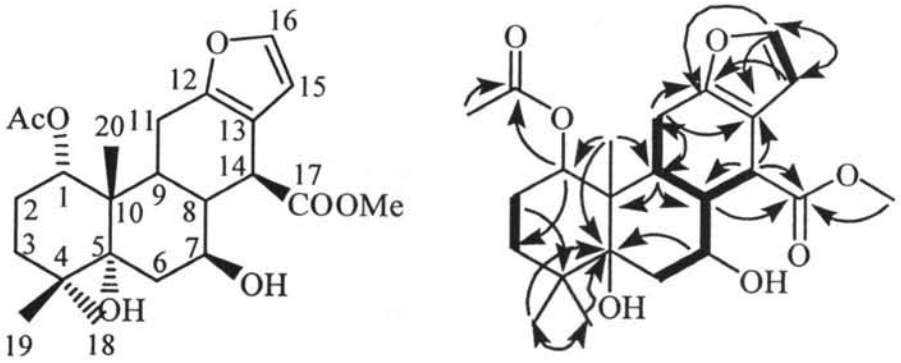

Figure 2.12 Key HMBC and COSY correlations for compound **12**

Table 2.12 The ^{1}H and ^{13}C NMR spectral data of Bonducellpin C and compound **12**

Position	Bonducellpin C		Compound 12	
	δ_C	δ_H (multiplicity *J* in Hz)	δ_C	δ_H (multiplicity *J* in Hz)
1	75.5	4.90 brs	75.6	4.91 brs
2	22.5	1.78 m	22.5	1.78 m
		1.99 m		1.99 m
3	30.0	1.15 m	29.9	1.15 m
		1.74 m		-
4	38.4	-	38.4	-
5	78.5	-	78.5	-
6	36.2	1.65 dd (13.6,11.3,2.8)	36.1	1.65 ddd (13.6,11.3,2.8)
		2.02 m		2.06 m
7	73.4	4.00 m	73.4	4.01 m
8	42.4	2.23 m	42.4	2.25 m
9	36.5	2.61 ddd (12.8,12.8,6.4)	36.5	2.61 ddd (12.8,12.8,6.4)
10	43.6	-	43.6	-
11	21.5	2.26 dd (16,12.8)	21.5	2.30 dd (16,12.8)
		2.50 ddd (16,6.4,3.2)		2.50 ddd (16,6.4,3.2)
12	150.0	-	150.0	-
13	113.6	-	113.7	-
14	46.4	3.47 d (8.8)	46.4	3.48 d (8.8)
15	108.5	6.17 d (2.5)	108.6	6.17 d (2.5)
16	141.4	7.24 d (2.5)	141.4	7.24 d (2.5)
17	176.0	-	176.1	-
18	28.0	1.05 s	27.9	1.06 s
19	25.0	1.09 s	25.0	1.10 s
20	17.7	1.19 s	21.4	1.20 s
1-OCO$\underline{C}H_3$	21.5	2.10 s	17.7	2.09 s
1-O$\underline{C}OCH_3$	169.0	-	169.0	-
OMe		3.74 s		3.75 s

Compound **13** was obtained as a colorless amorphous solid. The ^{1}H and ^{13}C NMR spectra (**Table 2.13**) were also closely resembled to those of Bonducellpin C (**12**), except for the disappearance of signals due to a carbomethyoxy substituent assigned to C-17 and appearance of signals due to one secondary methyl (δ_H 1.11). The secondary methyl was determined to be located at C-14 by the COSY and HMBC spectral analysis (**Figure 2.13**). Comparison of the ^{1}H and ^{13}C NMR spectral data to those published in the literature (**Table 2.13**) confirmed that compound **13** was Caesalpinin K, previously isolated from *Caesalpinia crista* of Indonesia.[41]

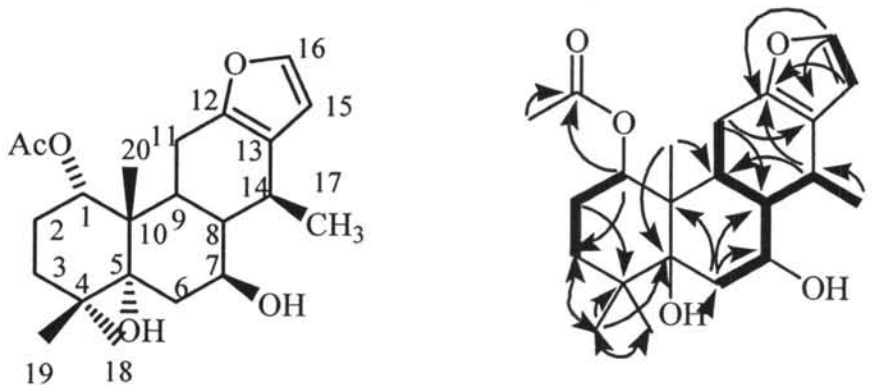

Figure 2.13 Key HMBC and COSY correlations for compound **13**

Table 2.13 The ^{1}H and ^{13}C NMR spectral data of Caesalpinin K and compound **13**

Position	Caeselpinin K		Compound 13	
	δ_C	δ_H (multiplicity *J* in Hz)	δ_C	δ_H (multiplicity *J* in Hz)
1	75.9	4.90 t (2.7)	75.8	4.91 t (2.7)
2	22.5	1.94 m	22.4	1.76 m
		1.73 m		1.96 m
3	30.0	1.15 m	29.9	1.16 m
		1.74 m		1.77 m
4	38.5	-	38.4	-
5	78.6	-	78.6	-
6	35.4	1.63 ddd (13.1,10.7,2.7)	35.3	1.64 ddd (13.1,10.7,2.7)
		2.12 dd (13.1,5.7)		2.12 dd (13.1,5.7)
7	67.8	4.20 td (10.7,5.7)	67.8	4.20 td (10.7,5.7)
8	42.6	2.77 m	42.5	-
9	31.9	2.67 ddd (12.1,10.5,6.7)	31.8	2.66 ddd (12.1,10.5,6.7)
10	43.5	-	43.4	-
11	22.0	2.28 dd (16.5,6.7)	22.0	2.28 dd (16.5,6.7)
		2.43 td (16.5,10.5)		2.43 td (16.5,10.5)
12	148.4	-	148.7	-
13	122.2	-	122.1	-
14	27.4	3.10	27.3	3.11
15	109.8	6.21 d (1.7)	109.8	6.22 d (1.7)
16	140.8	7.23 d (1.7)	140.7	7.24 d (1.7)
17	17.1	1.11 d (6.4)	17.1	1.11 d (6.4)
18	28.1	1.07 s	28.1	1.11 s
19	25.2	1.11 s	25.2	1.07 s
20	17.8	1.03 s	17.7	1.14 s
1-OCO<u>CH</u>$_3$	21.5	2.10 s	21.5	2.10 s
1-O<u>C</u>OCH$_3$	169.0	-	169.0	-
5-OH		2.98 d (2.7)		2.99 d (2.7)

Compound **14** was obtained as a colorless amorphous solid. The ^{1}H and ^{13}C NMR spectra (**Table 2.14**) were also similar to those of Bonducellpin C (**12**), except for the disappearance of the methoxyl ester and the significant difference was the downfield shift of the C-7 proton by 1.04 ppm in the ^{1}H NMR spectrum of compound

12. This suggested that the carbonyl ester at C-17 was converted to a lactone by cyclization of a carboxyl at C-17 and a hydroxyl at C-7. Thus, compound **14** was assumed to be Caesalmin B, which was confirmed by the COSY and HMBC spectra analysis (**Figure 2.14**) and by comparison of the NMR spectral data to those published in the literature (**Table 2.14**). Caesalmin B was also previously isolated from *Caesalpinia minax*[42] and *Caesalpinia crista* of Indonesia.[41]

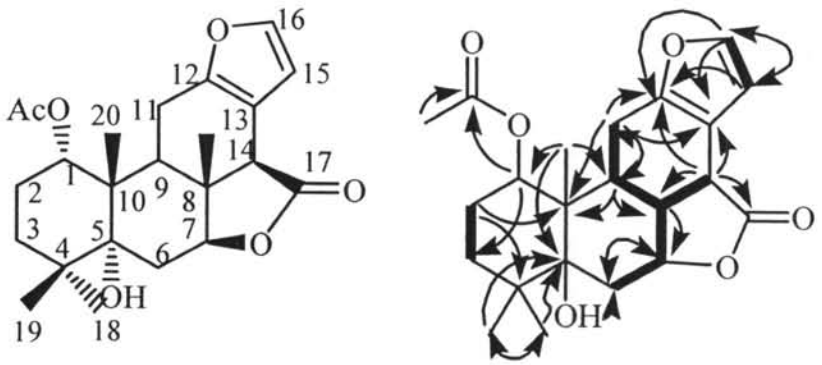

Figure 2.14 Key HMBC and COSY correlations for compound **14**

Table 2.14 The ^{1}H and ^{13}C NMR spectral data of Caesalmin B and compound **14**

Position	Caesalmin B		Compound 14	
	δ_C	δ_H (multiplicity *J* in Hz)	δ_C	δ_H (multiplicity *J* in Hz)
1	75.1	4.90 brs	74.6	4.95 brs
2	30.6	2.03 m	22.4	1.72 m
3	30.9	1.17 m	30.0	1.25 m
4	39.6	-	39.2	-
5	82.5	-	82.1	-
6	23.0	1.28 m	30.1	1.78 m
		1.96 m		2.4 m
7	82.0	4.77 dd (11.3,9.4)	80.7	4.7 dd (11.3,9.4)
8	45.5	2.09 m	46.4	1.97 m
9	34.0	2.82 td (13.5,8.8)	33.3	2.81 td (13.5,8.8)
10	47.3	-	44.8	-
11	21.6	2.53 m	21.3	2.53 m
12	152.7	-	151.5	-
13	114.7	-	114.0	-
14	41.7	3.30 d (13.5)	41.3	3.23 d (13.5)
15	107.9	6.59 d (1.9)	107.9	6.61 d (1.9)
16	142.2	7.30 d (1.9)	141.7	7.3 d (1.9)
17	176.6	-	174.6	-
18	28.1	1.13 s	28.1	1.09 s
19	24.4	1.18 s	24.6	1.12 s
20	17.3	1.22 s	17.4	1.14 s
1-OCO$\underline{CH_3}$	20.61	2.16 s	21.3	2.11 s
1-O$\underline{C}$OCH$_3$	170.6	-	168.7	-

Compound **15** was obtained as a colorless amorphous solid. The ^{1}H and ^{13}C NMR spectra (**Table 2.15**) were very similar to those of Caesalmin B (**14**), except for the presence of keto carbonyl at C-1 (δ_C 212.5) instead of the acetyl group in compound **14**. In addition, the acetyl group was located at the C-6 (δ_H 5.46) as showed HMBC cross peaks to C-5, C-7, C-8, and the acetyl carbon (δ_C 168.6) (**Figure 2.15**). Compound **15** was thus assumed to be Caesalpinin I confirmed by the analysis of ^{1}H-^{1}H COSY and HMBC correlations (**Figure 2.15**) and by comparison of the NMR spectral data of those published in the literature (**Table 2.15**). Caesalpinin I was previously isolated from *Caesalpinia crista* of Indonesia.[41]

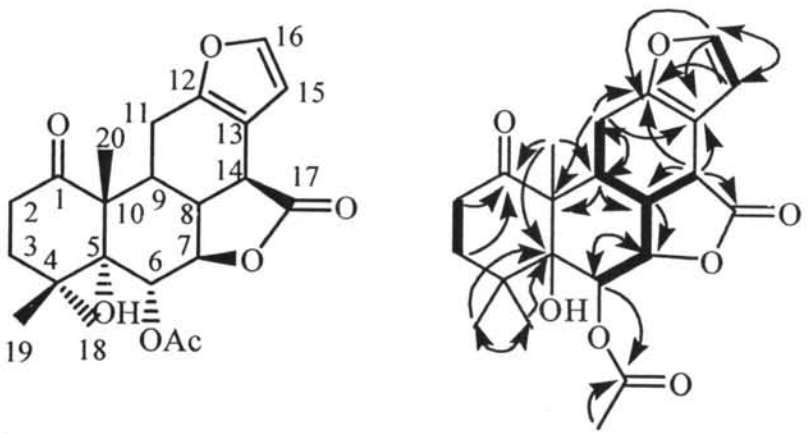

Figure 2.15 Key HMBC and COSY correlations for compound **15**

Table 2.15 The ^{1}H and ^{13}C NMR spectral data of Caesalpinin I and compound **15**

Position	Caesalpinin I		Compound 15	
	δ_C	δ_H (multiplicity *J* in Hz)	δ_C	δ_H (multiplicity *J* in Hz)
1	212.5	-	212.5	-
2	34.5	2.63 ddd (17.9,13.7,5.4) 2.43 dt (17.9,4.3)	34.5	2.63 ddd (17.9,13.7,5.4) 2.46 dt (17.9,4.3)
3	36.5	2.06 m 1.65 m	36.4	2.09 m 1.64 m
4	38.7	-	38.7	-
5	84.0	-	84.0	-
6	73.9	5.45 d (9.2)	73.9	5.46 d (9.2)
7	82.3	4.52 dd (11.8,9.2)	82.3	4.52 dd (11.8,9.2)
8	44.4	2.10 m	44.3	2.06 m
9	34.5	2.84 m	34.4	2.85 m
10	58.3	-	58.3	-
11	24.4	3.33 m 2.77 m	24.4	3.29 m 2.76 m
12	152.9	-	152.9	-
13	112.6	-	112.6	-
14	41.2	3.31 d (13.2)	41.1	3.30 d (13.2)
15	107.5	6.58 d (1.9)	107.5	6.58 d (1.9)
16	141.6	7.29 d (1.9)	141.6	7.28 d (1.9)
17	173.2	-	173.2	-
18	27.6	1.22 s	27.6	1.30 s
19	27.3	1.30 s	27.3	1.23 s
20	14.3	1.39 s	14.3	1.39 s
6-OCO$\underline{C}H_3$	21.4	2.18 s	21.4	2.14 s
6-O$\underline{C}OCH_3$	168.6	-	168.7	-
5-OH		2.47 s		2.5 s

Compound **16** was obtained as a white crystal. The ^{1}H and ^{13}C NMR spectra (**Table 2.16**) were similar to those of 14(17)-Dehydrocaesal F (**10**), but showed the characteristic signal of an aromatic proton (δ_H 7.05) and an aromatic-substituted tertiary methyl (δ_H 2.39) accompanied by the disappearance of an exomethylene at C-14, C-17. Thus, compound **16** was assumed to be 2-acetoxycaesaldekarin e, which was confirmed by the analysis of COSY and HMBC spectra. Comparison of the ^{1}H and ^{13}C NMR spectral data was also confirmed that compound **16** was 2-Acetoxycaesaldekarin e, previously obtained from *Caesalpinia crista* of Myanmar[43] and semi-synthesis of caesalpin F.[44]

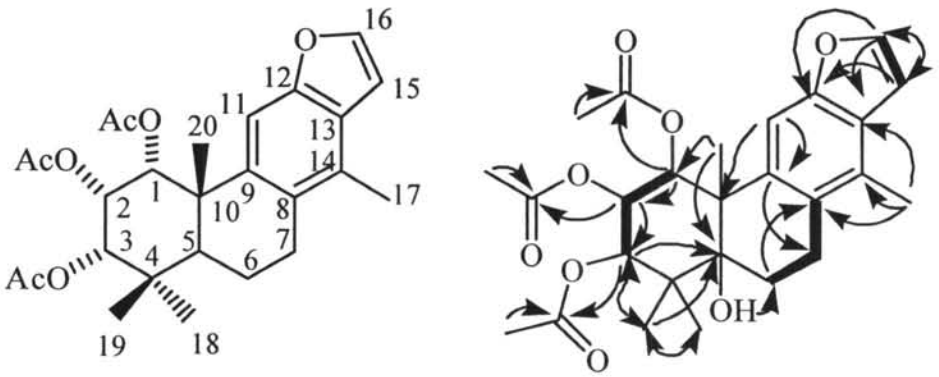

Figure 2.16 Key HMBC and COSY correlations for compound **16**

Table 2.16 The ^{1}H and ^{13}C NMR spectral data of compound **16**

Position	Compound 16		Position	Compound 16	
	δ_C	δ_H (multiplicity *J* in Hz)		δ_C	δ_H (multiplicity *J* in Hz)
1	73.7	6.05 brs	14	128.6	-
2	66.3	5.73 m	15	104.9	6.72
3	77.2	5.27 m	16	153.4	7.51 s
4	43.0	-	17	16.0	2.39 s
5	75.6	-	18	23.0	1.23 s
6	23.5	2.84 m	19	25.2	1.32 s
		2.94	20	31.2	1.52 s
7	24.4	2.11 m	1-OCO$\underline{C}H_3$	20.7	2.05
8	127.4	-	1-O$\underline{C}OCH_3$	169.6	-
9	139.7	-	2-OCO$\underline{C}H_3$	21.0	1.96
10	48.5	-	2-O$\underline{C}OCH_3$	169.7	-
11	104.1	7.05	3-OCO$\underline{C}H_3$	20.8	2.08
12	144.4	-	3-O$\underline{C}OCH_3$	170.0	-
13	125.7	-			

Compound **17** was isolated as a colorless amorphous solid (m.p.150-152^{0}C) and its molecular formular, $C_{24}H_{32}O_8$, was established by EIMS. The UV spectrum of compound **17** in CH_2Cl_2 at 230 nm. IR adsorptions at 3522, 1749 and 1713 cm^{-1} indicated the presence of hydroxyl, ketone carbonyl, and ester carbonyl, respectively. The ^{1}H NMR spectrum (**Table 2.17**) displayed the characteristic signals corresponding to four tertiary methyls, two oxymethines, two aliphatic methines, and two acetyl methyls. The 1,2-disubstituted furan ring was evident from the low-field doublets at δ_H 7.20 and 6.35. Moreover, the ^{13}C NMR and HSQC spectra showed 24 nonequivalent carbons for four olefinic carbons (δ_C 149.1, 141.6, 124.3, 107.1), four oxygen-substitued carbons (δ_C 82.8, 79.4, 73.1, 72.9), two ester carbonyl carbons (δ_C 170.8, 169.8), and one ketone carbonyl carbon (δ_C 211.6). Analysis of the COSY spectrum led to the partial structures depicted by the bold lines, which were connected

on the basis of the long-range correlations observed in the HMBC spectrum shown by arrow shown in **Figure 2.17**. The locations of acetyl groups was assigned to be C-6 and C-7 due to HMBC correlations of H-6 (δ_H 5.57) with the ester carbonyl carbon at δ_C 168.9 and H-7 (δ_H 5.58) with ester carbonyl carbon at δ_C 170.8. In addition, the ketone group was located at C-1 as showed HMBC cross peaks to H_2-2, H_2-3, and H_3-20. The relative stereochemistry of compound **J** was established by analysis of the NOESY spectrum. The observed NOE between H-7 and Me-17 and the lack of NOE correlation between H-8 and Me-17 and between H-8 and H-7 suggested that Me-17 and H-7 were α-oriented as shown in **Figure 2.18**. On the basis of the above the spectroscopic studies, compound J was thus identified as a new compound and has been given name Caesalpinin Q.

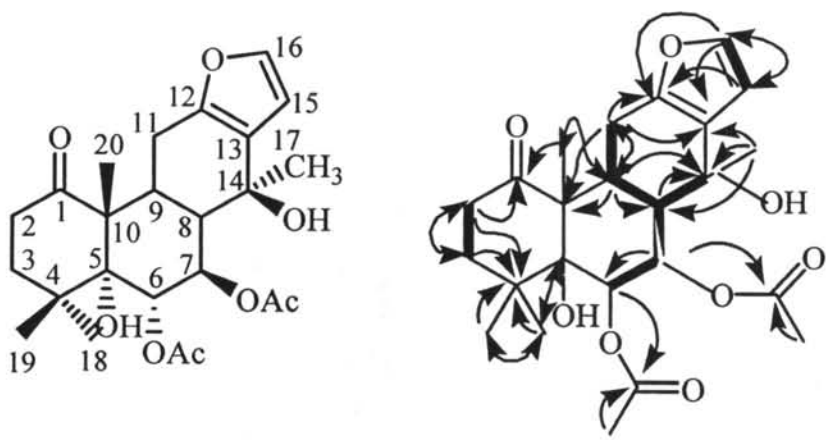

Figure 2.17 Key HMBC and COSY correlations for compound **17**

Table 2.17 The ^{1}H and ^{13}C NMR spectral data of compound **17**

Position	Compound 17	
	δ_C	δ_H (multiplicity J in Hz)
1	211.6	-
2	39.4	1.91 m
		1.73 m
3	35.2	2.77 m
		2.32 m
4	38.8	-
5	82.8	-
6	74.9	5.57 m
7	73.1	5.58 m
8	48.1	2.10 m
9	36.8	2.80 m
10	55.0	-
11	25.2	3.14 dd (16.4,5.2)
		2.43 dd (16.4,5.2)
12	149.1	-
13	124.3	-
14	72.9	-
15	107.1	6.35 d (1.6)
16	141.6	7.20 d (1.6)
17	24.5	1.49 s
18	29.0	1.14 s
19	25.6	1.33 s
20	16.4	1.52 s
6-OCO<u>CH</u>$_3$	21.4	2.09 s
6-O<u>C</u>OCH$_3$	169.8	-
7-OCO<u>CH</u>$_3$	21.4	2.00 s
7-O<u>C</u>OCH$_3$	170.8	-

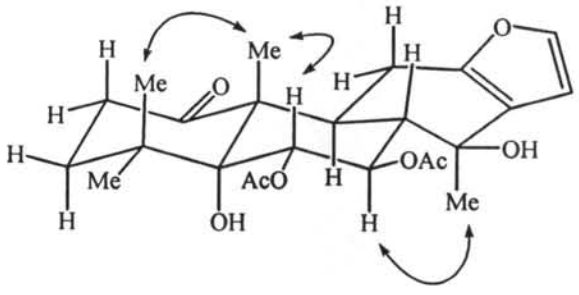

Figure 2.18 Selected NOE correlations for compound **17**