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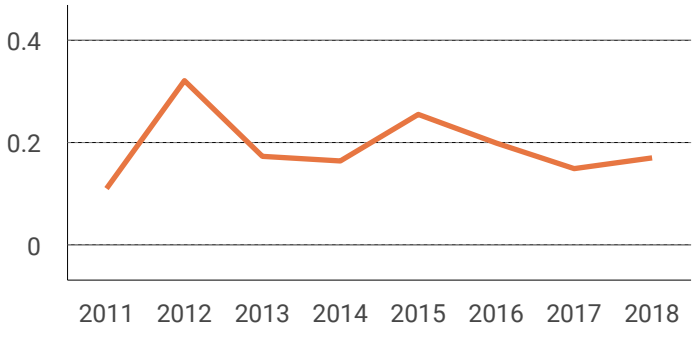

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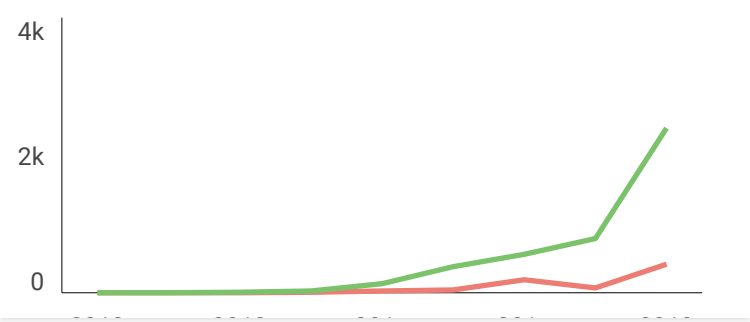

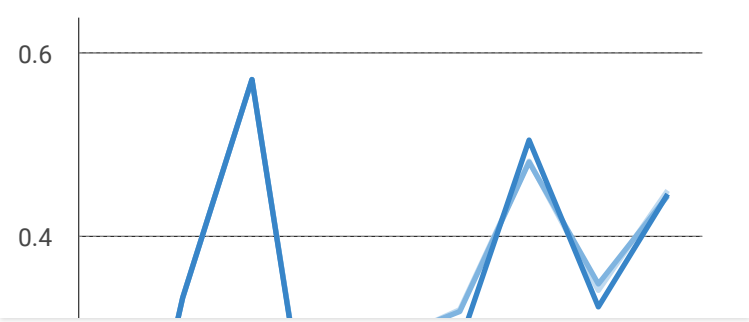

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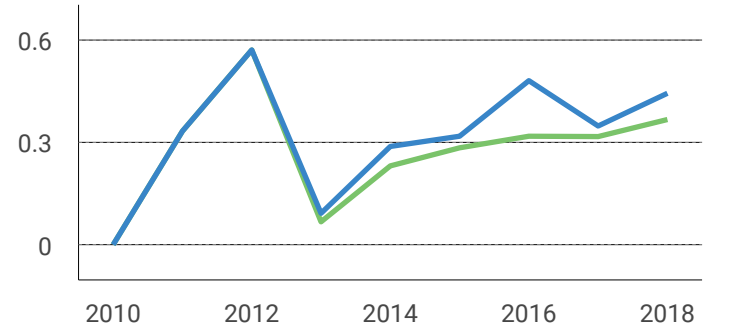

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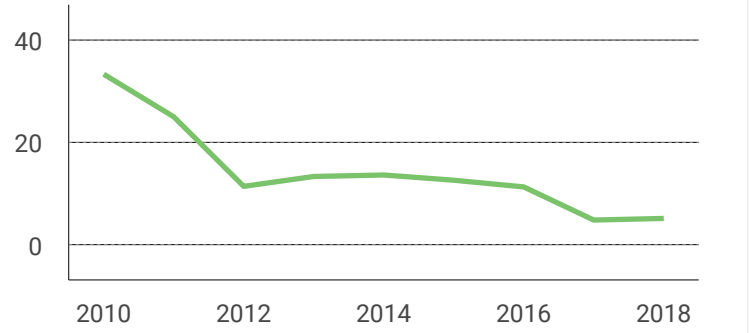

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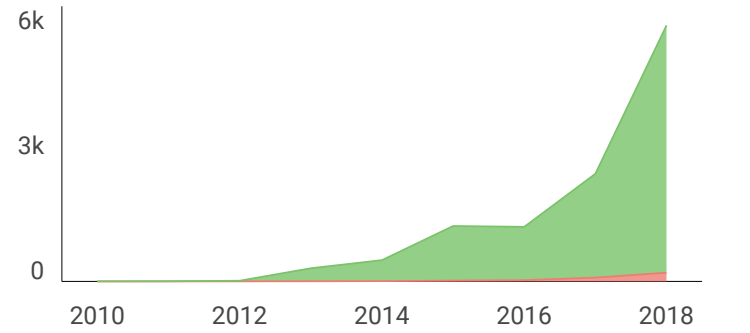

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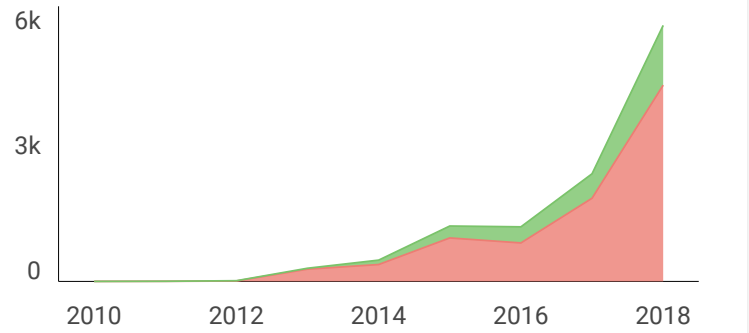

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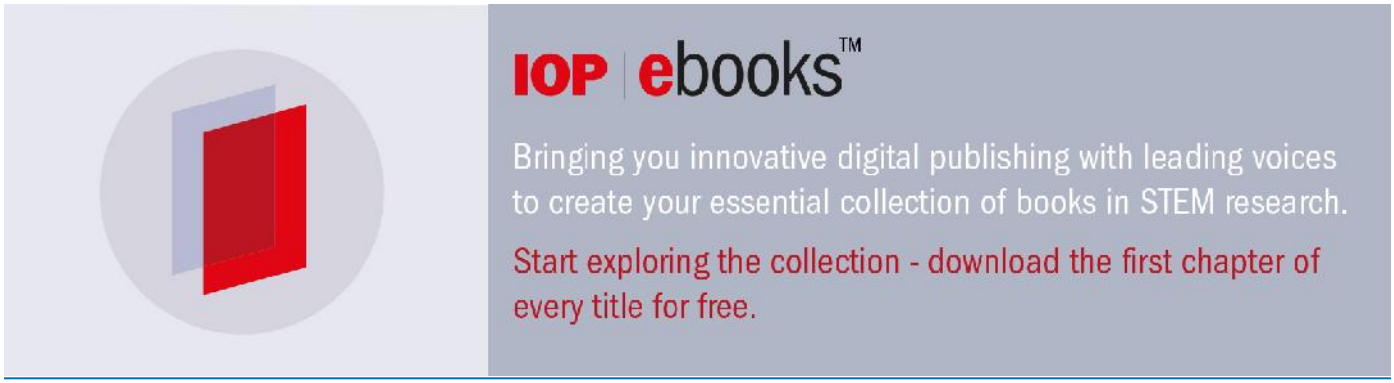

Chromanone Acid Derivatives from the Stem Bark of *Calophyllum incrassatum*

U Hasanah[1], T S Tjahjandarie[1], M Tanjung[1]
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Abstract. Two chromanone acid derivatives, calofolic acid B (**1**) and apetalic acid (**2**) were isolated from the stem bark of *Calophyllum incrassatum*. The structures of both compounds were elucidated by UV, IR, HRESIMS, 1D and 2D NMR spectroscopies and comparison of their data with literatures. Compounds **1-2** were evaluated for their cytotoxic activity against P-388 cells, compound (**1**) showing value IC_{50} 1.14 µg/mL.

Keywords: *Calophyllum incrassatum,* Calofolic acid B, Apetalic acid, P-388 cells.

1. Introduction

The genus *Calophyllum* (Calophyllaceae) comprises approximately 150 species that grow in the restrictive area of South East Asia. In Indonesia, the plants has many advantages, used as traditional treatment for rheumatism, inflammation, eye disease, and skin disease [1]. This genus is well known to produce a variety secondary metabolites such as xanthones [2], 4-phenylcoumarins [3], benzofuran-3-ones [4], biflavonoids [5] and chromanone acid [6-8], which have some biological activities such as anticancer, antibactery, and antitumor. Xanthone and 4-phenylcoumarin derivatives containing a isoprenyl side chain in aromatic region revealed as a key element to enhance their biological activities. In continuation of our work on phytochemical examination of chromanone acid on *Calophyllum* from West Kalimantan, we report the isolation of calofolic acid B (**1**), and apetalic acid (**2**) from the methanol extract of the stem bark of *Calophyllum incrassatum*. Compounds **1** and **2** were established by UV, IR, HRESIMS, 1D and 2D NMR, as well as by comparison with those related compounds previously reported. The cytotoxic activity against murine leukemia P-388 cells of isolated compounds from this species is also briefly described.

2. Experimental Methode

2.1 General experimental

UV spectra was measured by Shimadzu series 1800 UV-VIS spectrophotometer with methanol (Kyoto, Japan). IR spectra was recorded in KBr on a One Perkin Elmer instrument (Waltham, MA, USA). Mass spectra was recorded on Synapt G2 mass spectrometer (Manchester, UK). NMR spectra (^{1}H-NMR, ^{13}C-NMR, HMQC, and HMBC) was obtained with a JEOL JNM-ECA 400 MHz FTNMR spectrophotometer (Tokyo, Japan) with $CDCl_3$ as the solvent and internal standard. Column chromatography and planar radial chromatography was carried out using Si gel 60 Cat. No. 1.07734.1000 and Si gel 60 PF_{254} No. 1.07749.1000 (Merck, Darmstadt, Germany).

2.2 Plant Material

The stem bark of *C. incrassatum* was collected from Mandor sub-district, Landak regency, West Kalimantan, Indonesia. The sampel was identified by Mr. Ismail Rachman from the Herbarium Bogoriense, Bogor, Center of Biological Research and Development.

2.3 Extraction and isolation

The dried stem bark of *C. incrassatum* (3.0 kg) was macerated in methanol twice at room temperature for 24 h. The solvent was evaporated under reduced pressure to obtain a dark brown residue (280 g). The methanol extract was then partitioned with *n*-hexane and ethyl acetate. The ethyl acetate fraction (43 gr) was subjected to vacum liquid chromatography over silica gel and eluted with *n*-hexane-ethyl acetate (from 9:1 to 7:3) to give fractions A-C. Fraction C (5.44 g) was applied to column chromatography and eluted with *n*-hexane-ethyl acetate (from 9:1 to 8:2) to produce subfractions C_1-C_2. Subfraction C_2 (1.2 g) was purified by planar radial chromatography using *n*-hexane-chloroform (from 9:1 to 3:7) yielded compound **1** (76 mg).

The *n*-hexane fraction (30 gr) was subjected to vacum liquid chromatography over silica gel and eluted with *n*-hexane-ethyl acetate (from 9:1 to 8:2) to give fractions D and E. Fraction D (20 g) was refractionated using column chromatography and eluted with *n*-hexane-ethyl acetate (from 9:1 to 8:2) gave sub-fractions D_1-D_2. Subfraction D_1 (2.84 g) was purified by planar radial chromatography using *n*-hexane-ethyl acetate (9:1) to yielded sub-fraction D_{11}-D_{13}. Sub-fraction D_{12} (487 mg) was purified by planar radial chromatography using *n*-hexane- ethyl acetate (9:1) to give D_{121}-D_{125}. Subfraction D_{125} (227.2 mg) was purified by planar radial chromatography using *n*-hexane-ether (9:1) to yielded compound **2** (30 mg).

2.4 Cytotoxic assay

Compounds **1**–**2** were tested to *in vitro* evaluating against murine leukemia P-388 cells by using MTT colorimetric method as previously reported [9-11]. Artonin E was used as the positive control.

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3. Result and Discussion

Calofolic acid B (**1**), a yellow solid, m.p. 117-118 ^oC, showed a quasimolecular ion $[M+H]^-$ at *m/z* 359.1538 corresponding to the molecular formula $C_{20}H_{24}O_6$. The UV absorption at λ_{max} (nm) (log ε) = 225 (3.35), 274 (3.89), 289 (3.27), 305 (3.33), 312 (3.35), and 338 (2.60) typical for a chromanone acid [5]. The IR spectrum of **1** indicated absorptions for hydroxyl (3423 cm^{-1}), conjugated carbonyl (1620 cm^{-1}), aromatic (1577 and 1545 cm^{-1}) and ether (1188 cm^{-1}) groups, respectively. The ^1H NMR spectrum of **1** (Table 1) showed the presence of a 2.3-dimethylchromanone ring which represent by a pair of methine signals [δ_H 4.51 (1H, *dq, J* = 6.5 and 3.2 Hz) and 2.55 (1H, *dq, J* = 7.2 and 3.2 Hz)] and two proton signals of methyl [δ_H 1.35 (3H, *d, J* = 6.5 Hz) and δ_H 1.13 (3H, *d, J* = 7.3 Hz)], a chelated hydroxyl group (δ_H 12.37 (*s*, 5-OH), a dimethylpyrano ring group [δ_H 6.60 (1H, *d, J* = 10.0 Hz), δ_H 5.46 (1H, *d, J* = 10.0 Hz), and *gem* dimethyl group at δ_H 1.38 (3H, *s*), δ_H 1.45 (3H, *s*)], and an alkyl carboxylic group consist of a methine [δ_H 3.77 (1H, *m*)], a methylene [δ_H 2.67 (1H, *dd, J* = 7.4, 15.2 Hz), δ_H 2.82 (1H, *dd, J* = 8.3, 15.2 Hz)], and a methyl [δ_H 1.26 (3H, *d, J* = 7.2 Hz)]. The ^{13}C NMR spectrum of **1** showed 20 carbon signals and the structure of **1** were confirmed by HMQC and HMBC spectra (Figure 1). The ^{13}C NMR spectrum of **1** corresponding to the chromanone acid. The 1D and 2D NMR spectra data are consistent with the previous published data [8].

Apetalic acid (**2**), a yellow solid, m.p. 117-118 ^oC, $[\alpha]_D^{20}$ +56^o (*c* = 0,0005, MeOH). The UV spectrum λ_{max} (nm) (log ε) = 227 (3.39), 274 (3.96), 300 (3.41), 305 (3.39), 311 (3.41) and 336 (2.48) and IR spectrum gave absorptions of 3436, 1622, 1598, 1496, 1446 and 1157 cm^{-1} similar to those of **1.** The ^1H NMR spectrum of **2** very similar with **1** and the main difference is alkyl carboxylic group at C-10. The ^1H NMR spectrum of **2** (Table 1) showed the presence of a 2.3-dimethylchromanone ring consist of a pair of methine [δ_H 4.50 (1H, *dq, J* = 6.5 and 3.2 Hz) and 2.53 (1H, *dq, J* = 7.3 and 3.2 Hz)] and two proton signals of methyl [δ_H 1.35 (3H, *d, J* = 6.6 Hz) and δ_H 1.13 (3H, *d, J* = 7.2 Hz)], a chelated hydroxyl group (δ_H 12.39, *s*, 5-OH), a dimethylpyrano ring group [δ_H 6.60 (1H, *d, J* = 10.0 Hz), δ_H 5.45 (1H, *d, J* = 10.0 Hz), and *gem* dimethyl group δ_H 1.37 (3H, *s*), δ_H 1.44 (3H, *s*)], and an alkyl carboxylic group consist of a methine [δ_H 3.68 (1H, *m*)], three methylene [δ_H 2.66 (1H, *dd, J* = 6.9, 15.2 Hz), δ_H 2.82 (1H, *dd, J* = 8.7, 15.2 Hz), δ_H 1.54 (1H, *m*), δ_H 1.82 (1H, *m*), δ_H 1.19 (2H, *m*)], and a methyl [δ_H 0.85 (3H, *t, J* = 7.3 Hz)]. The ^{13}C NMR (Table.1) of **2** showed 22 carbon signals. The assignment of ^{13}C NMR spectrum was

confirmed by HMQC and HMBC spectra (Figure 1). The 1D and 2D NMR spectra data are consistent with those of published data [12].

Table-1. NMR spectroscopic data of compounds **1-2** in $CDCl_3$

No	Calofolic acid B (1)			Apetalic acid (2)		
	δ_H (mult, *J* in Hz)	**δ_C**	**HMBC**	**δ_H (mult, *J* in Hz)**	**δ_C**	**HMBC**
1	-	-	-	-	-	-
2	4.51 (*dq*, 3.2; 6.5)	76.1	C-4; C-16	4.50 (*dq*, 3.2; 6.5)	76.1	C-4, C-16
3	2.55 (*dq*, 3.2; 7.2)	44.2	C-4; C-16	2.53 (*dq*, 3.2; 7.3)	44.3	C-4; C-16
4	-	201.1	-	-	201.1	-
5	-	157.2	-	-	157.4	-
6	6.60 (*d*, 10.0)	115.7	C-8; C-13; C-14	6.60 (*d*, 10.0)	115.7	C-8; C-13; C-14
7	5.46 (*d*, 10.0)	125.8	C-8; C-13; C-17; C-18	5.45 (*d*, 10.0)	125.7	C-8; C-13; C-17; C-18
8	-	78.2	-	-	78.2	-
9	-	-	-	-	-	-
10	-	110.7	-	-	108.9	-
11	-	159.4	-	-	157.4	-
12	-	101.4	-	-	101.3	-
13	-	102.7	-	-	102.6	-
14	-	159.7	-	-	158.9	-
15	1.35 (*d*, 6.5)	16.2	C-2; C-3	1.35 (*d*, 6.6)	16.3	C-2; C-3
16	1.13 (*d*, 7.3)	9.4	C-2; C-3; C-4	1.13 (*d*, 7.2)	9.4	C-2; C-3; C-4
17	1.38 (*s*)	28.1	C-7; C-8; C-18	1.44 (*s*)	28.6	C-7; C-8; C-18
18	1.45 (*s*)	28.5	C-7; C-8; C-17	1.37 (*s*)	28.1	C-7; C-8; C-17
19	3.77 (*m*)	25.6	C-10; C-14; C-20; C-22	3.68 (*m*)	30.2	C-10; C-14; C-20; C-21; C-22
20	2.67 (*dd*, 7.4;	39	C-10; C-19; C-	2.66 (*dd*, 6.9; 15.2)	38	C-10; C-19;

	15.2) 2.82 (*dd*, 8.3; 15.2)	.5	21; C-22	2.82 (*dd*, 8.7; 15.2)	.6	C-21; C-22
21	-	179.6	-	-	179.5	-
22	1.26 (*d*, 7.2)	19.3	C-10; C-19; C-20	1.54 (*m*) 1.82 (*m*)	35.5	C-10; C-19; C-20; C-23; C-24
23	-	-	-	1.19 (*m*)	20.8	C-19; C-22; C-24
24	-	-	-	0.85 (*t*, 7.3)	14.1	C-22; C-23
5-OH	12.37 (*s*)	-	C-5; C-12; C-13	12.39	-	C-5; C-12; C-13

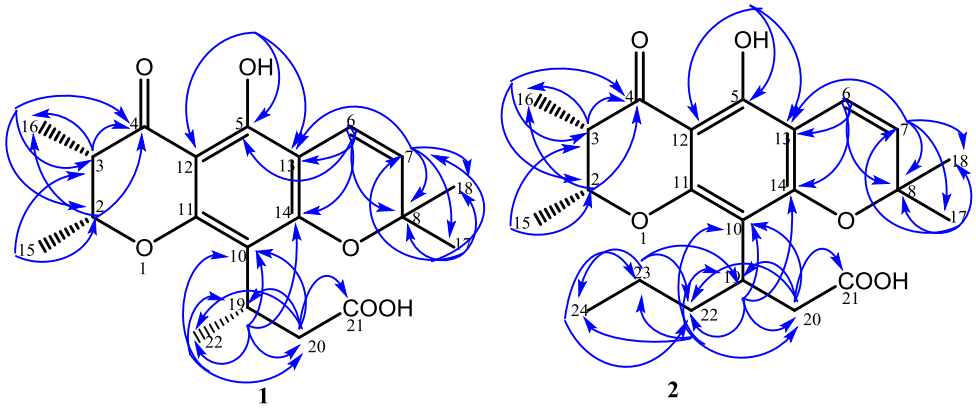

Figure 1. HMBC correlations for compounds **1** and **2**

The cytotoxic activity of compounds **1-2** was evaluated for their cytotoxicity by MTT assay against murine leukemia P-388 (Table. 2). Artonin E was used as the positive control. Those cytotoxic data for chromanone acid suggested that compound **1** has active and compound **2** was inactive.

Table-2. Cytotoxic activity data of compounds **1-2** by MTT assay.

No	**Compound**	**IC_{50} (µg/mL)**
1	Calofolic acid B (**1**)	1.14
2	Apetalic acid (**2**)	> 100
3	Artonin E	1.33

4. Conclusions

Chemical study on the stem bark of *C. incrassatum* yielded two chromanone acid derivatives, calofolic acid B (**1**) and apetalic acid (**2**). Calofolic acid B (**1**) showed very active against murine leukemia P-388 cells.

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