

Chromanone Derivatives from the Pericarps of *Calophyllum polyanthum*

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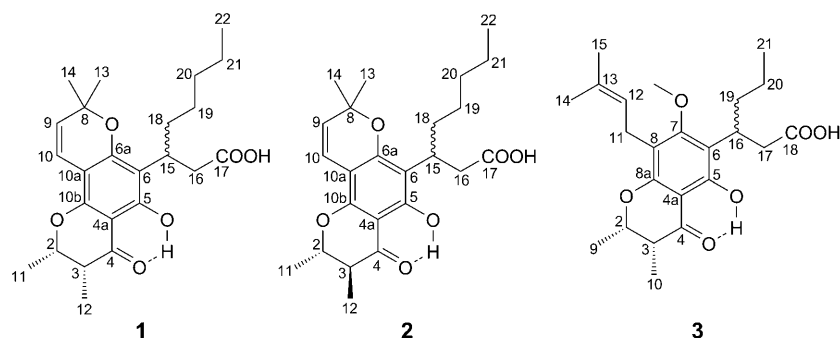
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Three new chromanone derivatives, calopolyanic acid (**1**), isocalopolyanic acid (**2**), and isorecedensic acid (**3**), were isolated from the pericarps of *Calophyllum polyanthum* WALL. ex CHOISY, along with seven known compounds, apetalic acid, blancoic acid, chapelieric acid, methyl isoapetalate, isoapetalic acid, isocalolongic acid, and recedensic acid. All of these compounds were reported from *C. polyanthum* for the first time. The structures of **1–3** were elucidated by spectroscopic methods.

Introduction. – The genus *Calophyllum* (Guttiferae) comprises ca. 130 species, mostly distributed in tropical areas of the world [1]. *Calophyllum* plants such as *C. brasiliense* [2][3], *C. inophyllum* [4][5], and *C. polyanthum* [6] are known to be rich in chromanone derivatives. Some of these compounds exhibit antiviral [3][7], antifungal [8], antibacterial [2][4], and cytotoxic activity [4][9]. In previous studies of *C. polyanthum*, some chromanone derivatives such as calopolyanolides C and D have been isolated [6][10]. We were interested in this type of constituents and conducted a phytochemical research on the pericarps of *C. polyanthum*, which led to the isolation of ten chromanone derivatives including three new ones, **1–3**. The structure elucidations of these new isolates are reported.

Results and Discussion. – Repeated chromatography over silica gel and *Sephadex LH-20*, and semipreparative HPLC on the AcOEt extract of the pericarps of *C. polyanthum* yielded three new chromanone derivatives, **1–3**.

Calopolyanic acid (**1**) was obtained as a yellow oil and assigned a molecular formula $C_{24}H_{32}O_6$ by HR-ESI-MS (m/z 415.2116 ($[M - H]^-$)), which indicated nine degrees of unsaturation. The UV data (λ_{max} 371, 314, 301, 277 nm) suggested the presence of a pyranochromanone moiety, which is common to chapelieric acid [11], isocalolongic acid [12], and other oxo-pyranochroman-carboxylic acids. The IR absorptions at $\tilde{\nu}_{max}$ 3433 and 1706 cm^{-1} indicated OH and CO groups, respectively. 1H - and ^{13}C -NMR data



(Table 1) pointed to the presence of an oxo-pyranochromane-carboxylic acid. The H-atom signals corresponding to two CH ($\delta(\text{H})$ 2.53 (*qd*, $J = 7.3, 3.1$), 4.56 (*qd*, $J = 6.5, 3.1$)) and two Me groups ($\delta(\text{H})$ 1.39 (*d*, $J = 6.5$), 1.18 (*d*, $J = 7.3$)) suggested the presence of the highly substituted 2,3-dimethylchromanone ring. In the $^1\text{H-NMR}$ spectrum, the two Me *singlets* at $\delta(\text{H})$ 1.44 (*s*) and 1.42 (*s*), and the two endocyclic olefinic *doublets* at $\delta(\text{H})$ 5.46 (*d*, $J = 10.0$) and 6.64 (*d*, $J = 10.0$) were attributable to a 2,2-dimethylpyrano ring fused to a benzene ring. These were further confirmed by the $^{13}\text{C-NMR}$ data (Table 1), $^1\text{H}, ^1\text{H-COSY}$ correlations, and HMBCs (Fig.). Based on the $^1\text{H}, ^1\text{H-COSY}$ correlations H–C(15)/CH₂(16), H–C(15)/CH₂(18), CH₂(18)/CH₂(19), CH₂(19)/CH₂(20), CH₂(20)/CH₂(21), and CH₂(21)/Me(22), and the HMBC cross-peak between H–C(15) and C(17), the presence of a 3-substituted octanoic acid unit was confirmed.

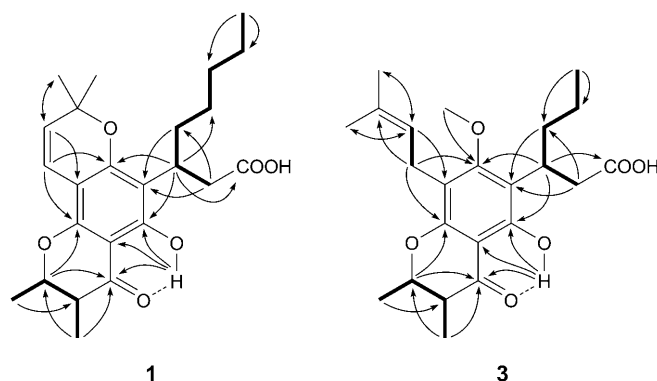


Figure. Key $^1\text{H}, ^1\text{H-COSY}$ (\longleftrightarrow) and HMBC (H \rightarrow C) correlations of **1** and **3**

The HMBCs (Fig.) H–C(9)/C(10a), H–C(10)/C(6a), and H–C(10)/C(10b) indicated the attachment of the dimethylpyrano moiety to C(6a) and C(10a) of the dimethylchromanone moiety. In the HMBC spectrum, diagnostic correlations H–C(15)/C(5), H–C(15)/C(6a), CH₂(16)/C(6), and CH₂(18)/C(6) confirmed that C(15) of the octanoic acid unit was attached to C(6) of the pyranochromanone moiety.

Table 1. ^1H - and ^{13}C -NMR Data of **1** and **2** (at 400 and 100 MHz, resp.; in CDCl_3 ; δ in ppm)

Position	1		2a		2b	
	$\delta(\text{H})$	$\delta(\text{C})$	$\delta(\text{H})$	$\delta(\text{C})$	$\delta(\text{H})$	$\delta(\text{C})$
2	4.56 (<i>qd</i> , $J=6.5$, 3.1)	76.0	4.16 (<i>dq</i> , $J=11.6$, 6.3)	78.7	4.16 (<i>dq</i> , $J=11.6$, 6.3)	78.7
3	2.53 (<i>qd</i> , $J=7.3$, 3.1)	44.3	2.56 (<i>dq</i> , $J=11.6$, 6.8)	45.6	2.56 (<i>dq</i> , $J=11.6$, 6.8)	45.6
4	–	200.8	–	198.7	–	198.7
4a	–	100.9	–	101.6	–	101.0
5	–	162.3	–	161.9	–	161.9
6	–	110.3	–	110.1	–	110.1
6a	–	160.0	–	159.4	–	159.4
8	–	78.0	–	77.91	–	77.94
9	5.46 (<i>d</i> , $J=10.0$)	125.7	5.47 (<i>d</i> , $J=9.8$)	125.7	5.47 (<i>d</i> , $J=9.8$)	125.7
10	6.64 (<i>d</i> , $J=10.0$)	115.8	6.54 (<i>d</i> , $J=9.8$)	115.8	6.54 (<i>d</i> , $J=9.8$)	115.8
10a	–	101.0	–	101.6	–	101.6
10b	–	155.0	–	155.1	–	155.1
11	1.39 (<i>d</i> , $J=6.5$)	16.5	1.50 (<i>d</i> , $J=6.3$)	19.7	1.50 (<i>d</i> , $J=6.3$)	19.7
12	1.18 (<i>d</i> , $J=7.3$)	9.5	1.21 (<i>d</i> , $J=6.8$)	10.0	1.21 (<i>d</i> , $J=6.8$)	10.1
13	1.44 (<i>s</i>)	28.3	1.47 (<i>s</i>)	28.5	1.43 (<i>s</i>)	28.3
14	1.42 (<i>s</i>)	28.3	1.39 (<i>s</i>)	28.0	1.43 (<i>s</i>)	28.2
15	3.62–3.74 (<i>m</i>)	30.4	3.63–3.74 (<i>m</i>)	30.4	3.63–3.74 (<i>m</i>)	30.4
16	2.81–2.89 (<i>m</i>), 2.77 (<i>dd</i> , $J=15.4$, 7.3)	38.0	2.84–2.93 (<i>m</i>), 2.74 (<i>dd</i> , $J=15.5$, 7.2)	38.0	2.84–2.93 (<i>m</i>), 2.76 (<i>dd</i> , $J=15.5$, 7.2)	37.9
17	–	178.4	–	177.6	–	177.6
18	1.78–1.92 (<i>m</i>), 1.52–1.62 (<i>m</i>)	32.9	1.80–1.94 (<i>m</i>), 1.54–1.63 (<i>m</i>)	32.9	1.80–1.94 (<i>m</i>), 1.54–1.63 (<i>m</i>)	32.9
19	1.20–1.21 (<i>m</i>), 1.10–1.14 (<i>m</i>)	27.5	1.21 (<i>d</i> , $J=6.8$), 1.04–1.14 (<i>m</i>)	27.42	1.21 (<i>d</i> , $J=6.8$), 1.04–1.14 (<i>m</i>)	27.38
20	1.21–1.28 (<i>m</i>)	31.8	1.24–1.28 (<i>m</i>)	31.8	1.24–1.28 (<i>m</i>)	31.8
21	1.21–1.28 (<i>m</i>)	22.6	1.22–1.24 (<i>m</i>)	22.6	1.22–1.24 (<i>m</i>)	22.6
22	0.82 (<i>t</i> , $J=6.3$)	14.0	0.82 (<i>t</i> , $J=6.5$)	14.1	0.82 (<i>t</i> , $J=6.5$)	14.1
Chelated OH	12.62 (<i>s</i>)	–	12.71 (<i>s</i>)	–	12.71 (<i>s</i>)	–

The HMBCs from the chelated OH group ($\delta(\text{H})$ 12.62) to C(4) and C(5) evidenced that the OH group was at C(5). These data suggested that the structure of **1** is similar to that of calolongic acid [8] and isocalolongic acid [12], except for the 3-substituted octanoic acid moiety at C(6) rather than a hexanoic acid unit.

The *cis*-configuration of H–C(2) and H–C(3) was established by the magnitude of the coupling constant ($J(2,3) = 3.1$ Hz) between the two H-atoms, as in isocalolongic acid. Therefore, the structure of compound **1**, named calopolonyanic acid, was determined as shown.

Isocalopolonyanic acid (**2**), yellow oil, was obtained as a pair of compounds, **2a** and **2b**, with a molecular formula $\text{C}_{24}\text{H}_{32}\text{O}_6$ deduced from the HR-ESI-MS data (m/z 415.2108 ($[M - \text{H}]^-$, $\text{C}_{24}\text{H}_{31}\text{O}_6^-$; calc. 415.2120). The ratio **2a/2b** was *ca.* 2:1 by the H-atom integration in the ^1H -NMR spectrum of **2**. According to the 1D- and 2D-NMR spectroscopic data, the C-atom connectivity of **2a** was found to be the same as that of **1**. The NMR data of **2a** (Table 1) showed major chemical-shift differences compared with those of **1** only for the two Me groups (Me(11) and Me(12)) and two CH groups (H–C(2) and H–C(3)) of the 2,3-dimethylchromanone ring. The coupling constant of

H–C(2) and H–C(3) ($J(2,3) = 11.6$ Hz) was remarkably different from that of **1** ($J(2,3) = 3.1$ Hz), revealing their different relative configuration, *i.e.*, *trans*, for **2a** as in pinetoric acid **1** [1] and calolongic acid [8]. Based on the 1D- and 2D-NMR analyses, the planar structure of **2b** was determined to be the same as that of **2a**. From the values of the coupling constant, H–C(2) and H–C(3) of both **2a** and **2b** were deduced as *trans*-configured, which implied that **2a** and **2b** might be a pair of *C(15)*-epimers. Therefore, the structure of compound **2**, named isocalopolyanic acid, was elucidated as depicted.

Isorecedensic acid (**3**), a yellow oil, had the molecular formula of $C_{23}H_{32}O_6$ with eight degrees of unsaturation as determined by HR-ESI-MS (m/z 403.2131 ($[M - H]^-$)). From the UV-, IR-, and NMR-spectroscopic data, identifiable structural constituents of **3** included the 2,3-dimethylchromanone ring and a 3-substituted hexanoic acid unit. In the 1H -NMR spectrum, a MeO signal was observed at $\delta(H)$ 3.74, and a low-field signal at $\delta(H)$ 12.28 was assignable to a chelated OH group (Table 2). The presence of a 3-methylbut-2-enyl moiety was deduced from the $^1H, ^1H$ -COSY cross-peaks and HMBCs (Fig.). The HMBC correlations from $CH_2(11)$ to C(7) and C(8a), and from H–C(12) to C(8) indicated the attachment of the 3-methylbut-2-enyl moiety to C(8) of the 2,3-dimethylchromanone unit. Correlations were also observed between H–C(16) and C(5), between H–C(16) and C(7), between $CH_2(17)$ and C(6), and between $CH_2(19)$ and C(6), indicating that the hexanoic acid unit was attached to C(6) through C(16). By HMBC experiment (Fig.), the MeO and OH groups were located at C(7) and C(5), respectively. These data suggested that the C-connectivity of **3** is the same as that of recedensic acid [12]. The relative configuration of C(2) and C(3) was also confirmed by the coupling constant of H–C(2) and H–C(3) ($J(2,3) = 3.3$ Hz), which indicated their *cis*-relationship. Therefore, the structure of isorecedensic acid (**3**) was elucidated as shown.

Table 2. 1H - and ^{13}C -NMR Data of **3** (at 400 and 100 MHz, resp., in $CDCl_3$; δ in ppm)

Position	$\delta(H)$	$\delta(C)$	Position	$\delta(H)$	$\delta(C)$
2	4.55 (<i>qd</i> , $J = 6.5, 3.3$)	75.8	11	3.26 (<i>dd</i> , $J = 14.7, 6.9$), 3.20 (<i>dd</i> , $J = 14.7, 6.9$)	22.8
3	2.60 (<i>qd</i> , $J = 7.3, 3.3$)	44.5	12	5.13 (<i>t</i> , $J = 6.9$)	123.1
4	–	202.2	13	–	131.2
4a	–	104.0	14	1.74 (<i>s</i>)	17.9
5	–	160.5	15	1.68 (<i>s</i>)	25.7
6	–	116.5	16	3.50–3.59 (<i>m</i>)	32.3
7	–	165.1	17	2.88 (<i>d</i> , $J = 7.2$)	37.8
7-OMe	3.74 (<i>s</i>)	61.8	18	–	178.3
8	–	113.4	19	1.78–1.91 (<i>m</i>), 1.64–1.71 (<i>m</i>)	35.4
8a	–	158.3	20	1.20–1.32 (<i>m</i>), 1.08–1.17 (<i>m</i>)	21.2
9	1.37 (<i>d</i> , $J = 6.5$)	16.2	21	0.87 (<i>t</i> , $J = 7.2$)	14.1
10	1.18 (<i>d</i> , $J = 7.3$)	9.3	Chelated OH	12.28 (<i>s</i>)	–

Comparing their NMR and MS data with those in the literature, the known compounds were identified as apetalic acid [9][13], blancoic acid [14], chapelieric acid [11], methyl isoapetalate [9][15], isoapetalic acid [9], isocalolongic acid [12], and recedensic acid [12]. All of them were reported from *C. polyanthum* for the first time.

Blancoic acid, methyl isoapetalate, isocalolongic acid, recedensic acid, and compounds **1**–**3** were evaluated for their biological activity against the K562 cell line as well as the fungus *Candida albicans*. However, none of them showed any activity.

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Experimental Part

General. Anal. TLC: pre-coated silica-gel- F_{254} plates (*Qingdao Meigao Chemical Co.*); spots were detected under UV light (254 and 365 nm), and by spraying with 5% aq. H_2SO_4 in EtOH, followed by heating. Column chromatography (CC): silica gel (SiO_2 , 80–100, 200–300, and 300–400 mesh; *Qingdao Meigao Chemical Co.*), *C-18* silica gel (40–75 μm ; *Fuji Silysia Chemical Ltd.*), *MCI* gel (70–150 μm ; *Mitsubishi Chemical Corporation*), and *Sephadex LH-20* gel (*GE Healthcare Bio-Sciences AB*). HPLC: *Agilent 1200*; semi-prep. column (*Zorbax SB-C₁₈*, 9.4 \times 250 mm, 5 μm); 2 ml/min. CF_3COOH (TFA): *Sinopharm Chemical Reagent Co. Ltd.* Optical rotations: *Horiba SEPA-300* polarimeter. UV Spectra: *Shimadzu double-beam 210A* spectrometer; λ_{max} (log ϵ) in nm. IR spectra: *Bio-Rad FTS-135* spectrophotometer; KBr pellets; in cm^{-1} . 1D- and 2D-NMR spectra: *BRUKER AM-400* and *DRX-500* spectrometers; δ in ppm rel. to Me_4Si , J in Hz. MS: *VG Auto Spec-3000* magnetic-sector instrument and *API Qstar Pulsar* instrument; in m/z .

Plant Material. The pericarps of *Calophyllum polyanthum* WALL. ex CHOISY were collected by Dr. Josef Margraf and Ms. Minguo Li from Xishuangbanna, Yunnan Province, P. R. China, in June 2008. The plant material was identified by C.-L. L., at the Kunming Institute of Botany, the Chinese Academy of Sciences (CAS). A voucher specimen (No. BN0801) was deposited with the Research Group for Biodiversity and Plant Resources, Kunming Institute of Botany, CAS, P. R. China.

Extraction and Isolation. The air-dried and powdered pericarps of *C. polyanthum* (9.0 kg) were exhaustively extracted with MeOH under reflux (9 h; 4, 3, and 3 h, resp.). The MeOH extract was evaporated under reduced pressure. The residue (1.5 kg) was suspended in H_2O (2 l) and extracted with petroleum ether (PE; 3 \times 2 l) and AcOEt (3 \times 2 l). The AcOEt-soluble portion (770 g) was chromatographed on silica-gel column with PE/ Me_2CO 5:1 to yield one fraction (3.9 g), which gave a large, yellowish spot on TLC (PE/ Me_2CO 2:1; R_f 0.6) after spraying with 5% aq. H_2SO_4 in EtOH and heating.

This fraction was subjected to CC (*Sephadex LH-20*; MeOH) to give four fractions, *Frs. 1–4*. *Fr. 2* (1.1 g) was fractionated by CC (SiO_2 ; PE/acetone 10:1) to afford four fractions, *Frs. 2a–2d*. *Fr. 2a* was rechromatographed on a *Sephadex LH-20* column (MeOH), followed by HPLC with a C_{18} semiprep. column with MeOH/ H_2O (containing 0.05% TFA; 85:15) to yield blancoic acid (t_R 30.1 min; 4 mg), methyl isoapetalate (t_R 31.4 min; 4 mg), and isoapetalic acid (t_R 19.7 min; 26 mg). *Fr. 2b* was separated by CC (*Sephadex LH-20*; MeOH), then by HPLC (MeOH/ H_2O , containing 0.05% TFA; 85:15) to yield recedensic acid (t_R 21.4 min; 5 mg) and **2** (t_R 23.5 min; 4 mg). In the same way, **1** (t_R 25.0 min; 5 mg), **3** (t_R 22.5 min; 6 mg), and apetalic acid (t_R 35.1 min; 6 mg) were obtained from *Fr. 2c*. *Fr. 3* (750 mg) was separated by CC (*Sephadex LH-20*; MeOH) into three major fractions, *Frs. 3a–3c*. Then, HPLC (MeOH/ H_2O , containing 0.05% TFA, 80:20) of *Fr. 3a* yielded isocalolongic acid (t_R 25.4 min; 10 mg), and *Fr. 3c* afforded chapelieric acid (t_R 27.3 min; 41 mg).

Calopolyanic Acid (= 3-[rel-(2R,3S)-3,4-Dihydro-5-hydroxy-2,3,8,8-tetramethyl-4-oxo-2H,8H-pyrano[2,3-f]chromen-6-yl]octanoic Acid; **1**). Yellow oil. $[\alpha]_D^{25} = -41.9$ ($c = 0.245$, $CHCl_3$). UV ($CHCl_3$): 371 (1.76), 314 (2.58), 301 (2.69), 277 (3.78). IR: 3433, 2929, 1706, 1628, 1592, 1458, 1198, 1135. 1H - and ^{13}C -NMR: Table 1. ESI-MS (neg.): 415 ($[M - H]^-$). HR-ESI-MS: 415.2116 ($[M - H]^-$, $C_{24}H_{31}O_6^-$; calc. 415.2120).

Isocalopolyanic Acid (= 3-[rel-(2R,3R)-3,4-Dihydro-5-hydroxy-2,3,8,8-tetramethyl-4-oxo-2H,8H-pyrano[2,3-f]chromen-6-yl]octanoic Acid; **2**). Yellow oil. $[\alpha]_D^{25} = +2.9$ ($c = 0.230$, $CHCl_3$). UV ($CHCl_3$):

373 (1.61), 313 (2.38), 300 (2.52), 277 (3.58), 234 (2.99), 193 (3.14). IR: 3433, 2929, 1707, 1628, 1595, 1460, 1189, 1123. ¹H- and ¹³C-NMR: Table 1. ESI-MS (neg.): 415 ([M – H][–]). HR-ESI-MS: 415.2108 ([M – H][–], C₂₄H₃₁O₆[–]; calc. 415.2120).

Isorecedensic Acid (= 3-*rel*-(2*R*,3*S*)-3,4-Dihydro-5-hydroxy-7-methoxy-2,3-dimethyl-8-(3-methylbut-2-en-1-yl)-4-oxo-2H-chromen-6-yl]hexanoic Acid; **3**). Yellow oil. [α]_D²² = –33.2 (c = 0.265, CHCl₃). UV (CHCl₃): 360 (1.38), 288 (2.67), 240 (2.16). IR: 3448, 2930, 1686, 1634, 1580, 1431, 1209, 1138. ¹H- and ¹³C-NMR: Table 2. ESI-MS (neg.): 403 ([M – H][–]). HR-ESI-MS: 403.2131 ([M – H][–], C₂₃H₃₁O₆[–]; calc. 403.2120).

Biological-Activity Assay. The inhibitory activities of blancoic acid, methyl isoapetalate, isocololonic acid, recedensic acid, and compounds **1–3** against the chronic myelogenous leukemia K562 cell line were evaluated by the MTT method [16], and against the fungus *Candida albicans* by the microdilution assay [17].

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