Two Pyranocoumarins from the Seeds of *Calophyllum polyanthum*

Chun-Hui Ma,[†,‡] Bin Chen,[†] Hua-Yi Qi,[†] Bo-Gang Li,[†] and Guo-Lin Zhang*[,†]

Chengdu Institute of Biology, the Chinese Academy of Sciences, Chengdu 610041, People's Republic of China, and Chengdu Institute of Organic Chemistry, the Chinese Academy of Sciences, Chengdu 610041, People's Republic of China

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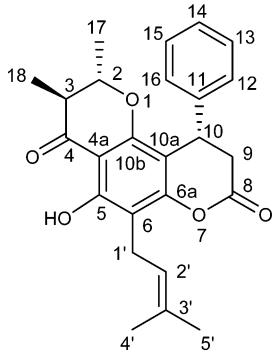
Two new pyranocoumarin diastereoisomers, calopolyanolides C (**1**) and D (**2**), were isolated from the ethanolic extract of the seeds of *Calophyllum polyanthum*, along with calopolyanolide A (**3**), calopolyanolide B (**4**), calanolide E2 (**5**), 5,7,3′,4′-tetrahydroxyisoflavone (**6**), 3,4-dihydroxybenzoic acid (**7**), 3,4,5-trihydroxybenzoic acid (**8**), 2-hydroxy-4-methoxybenzoic acid (**9**), *β*-sitosterol (**10**), *β*-daucosterol (**11**), 3,5-dihydroxy-4-methoxybenzoic acid (**12**), 3,5-dimethoxybenzoic acid (**13**), and ursolic acid (**14**). Their structures were determined by MS, UV, and IR data and HMQC, HMBC, and NOESY experiments or by comparing them with authentic samples. X-ray crystallographic analysis confirmed the structure and relative configuration of **1**.

The species of the genus *Calophyllum* (Guttiferae) are mainly distributed in tropical areas. *C. inophyllum* L., *C. membranaceum* Gardn. et Champ., *C. polyanthum* Wall. et Choisy, and *C. blancoi* Planch et Triana are found in China,[1] and plants from *Calophyllum* are known to be rich in coumarins[2−7] and xanthones.[8−12] Some pyranocoumarins, such as calanolide A, calanolide B, inophyllum B, and inophyllum P, have shown significant anti-HIV activity.[13,14]

From *C. polyanthum* Wall, the anti-HIV compound calanolide E2 and four other compounds have been isolated.[15] In the present study on *C. polyanthum*, two new pyranocoumarin diastereoisomers, calopolyanolide C (**1**) and calopolyanolide D (**2**), were isolated, together with 12 known compounds, calopolyanolide A (**3**), calopolyanolide B (**4**), calanolide E2 (**5**), 5,7,3′,4′-tetrahydroxyisoflavone (**6**), 3,4-dihydroxybenzoic acid (**7**), 3,4,5-trihydroxybenzoic acid (**8**), 2-hydroxy-4-methoxybenzoic acid (**9**), *β*-sitosterol (**10**), *β*-daucosterol (**11**), 3,5-dihydroxy-4-methoxybenzoic acid (**12**), 3,5-dimethoxybenzoic acid (**13**), and ursolic acid (**14**).



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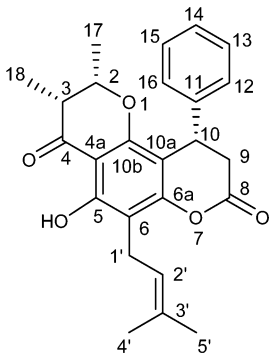

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Compound **1** was obtained as pale yellow needles. A molecular formula of $C_{25}H_{26}O_5$ was determined from the ion peak at *m*/*z* 405.1715 $[M - H]^-$ in the HRESIMS spectrum. The IR absorption at ν_{max} 3435 cm^{-1} and the ^1H NMR signal at δ 12.31 (1H, s) indicated the presence of a hydroxyl group. The IR peaks at ν_{max} 1780 and 1630 cm^{-1} and the ^{13}C NMR signals at δ 166.7 (C-8) and 199.9 (C-4) suggested a saturated lactone and a hydrogen-bonded carbonyl group.

The presence of a 3-methylbut-2-enyl moiety was deduced from the H−H COSY cross-peaks, H-1′ (δ 3.41 and 3.36, each 1H, dd, J = 14.1 Hz, 7.2 Hz)/H-2′ (δ 5.23, 1H, t, J = 6.6 Hz), and HMBC correlations H-2′/C-4′ (δ 18.0), C-5′ (δ 21.5) and H-4′ (δ 1.81, 3H, s), H-5′ (δ 1.68, 3H, s)/C-3′ (δ 132.7), C-2′ (δ 121.6).

The H−H COSY correlations H-17 (δ 1.42, 3H, d, J = 6.2 Hz)/H-2 (δ 4.22, 1H, dq, J = 12.2 Hz, 6.2 Hz), H-18 (δ 1.22, 3H, d, J = 6.8 Hz)/H-3 (δ 2.54, dq, J = 13.8 Hz, 7.0 Hz), and H-2/H-3 and the ^{13}C NMR signals at δ 19.6 (C-17), 10.6 (C-18), 79.7 (C-2), and 46.1 (C-3) assigned from HMQC data suggested the presence of the structural unit −CH(CH_3)−CH(CH_3)−O−.

The ^1H NMR signals at δ 7.28 (2H, t, J = 7.3 Hz, H-13 and H-15), 7.22 (1H, t, J = 7.3 Hz, H-14), and 7.12 (2H, d, J = 7.6 Hz, H-12 and H-16) suggested the presence of a monosubsituted phenyl group, which was located at C-10 by considering the H−H COSY correlations of H-10 (δ 4.56, 1H, d, J = 5.9 Hz)/H-9 (δ 3.05, 1H, dd, J = 15.9 Hz, 1.5 Hz; 2.99, dd, J = 15.9 Hz, 7.0 Hz) and HMBC correlations H-10/C-12, 16 (δ 126.9) and H-9/C-11 (δ 141.4).

In addition to the carbons of the structural units discussed above, six quaternary carbons (δ 156.4, 104.0, 156.2, 104.3, 160.5, and 110.6) remained and suggested the presence of a fully substituted phenyl ring (ring B). HMBC correlations H-10/C-10a (δ 104.0), C-10b (δ 156.2), C-6a (δ 156.4); H-9/C-10a; H-9, H-10/C-8; and H-2/C-10b confirmed the structure assigned to ring C. Definition of ring A was deduced from HMBC correlations of H-18, H-2/C-4 and H-2/C-10b. The 3-methylbut-2-enyl moiety was located at C-6 by the HMBC correlations H-1′/C-6a, C-5 (δ 160.5) and H-2′/C-6. The HMBC correlations arising from the hydroxyl group at δ_H 12.31 with C-4a (δ 104.4), C-5, and C-6 (δ 110.6) indicated that the hydroxyl group (5-OH) should be located at C-5 and intramolecularly hydrogen bonded to the carbonyl group at C-4 (Figure 1).[16]

The coupling constant between H-2 and H-3 (12.2 Hz) in **1** suggested a trans diaxial orientation of H-2 and H-3. NOESY cross-peaks between H-17/H-3, H-16 revealed an α oriention of the phenyl moiety. Accordingly, the structure of compound **1** was defined as 5-hydroxy-2α,3*β*-dimethyl-6-(3-methylbut-2-enyl)-10α-phenyl-2,3,9,10-tetrahydropyrano[2,3-*f*]chromen-4,8-dione (calopolyanolide C). X-ray crystallographic analysis further served to confirm the elucidation of structure **1** (Figure 2).

* Corresponding author. Tel/Fax: +86-28-85225401. E-mail: zhanggl@cib.ac.cn.
† Chengdu Institute of Biology.
‡ Chengdu Institute of Organic Chemistry.

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Table 1. ^{1}H ($CDCl_3$, 600 MHz) and ^{13}C ($CDCl_3$, 150 MHz) NMR data of Compounds **1** and **2**[a]

	1			**2**	
position	δ_C	δ_H (int., mult., *J* in Hz)	position	δ_C	δ_H (int., mult., *J* in Hz)
2	79.7	4.22 (1H, dq, 12.2, 6.2)	2	77.0	4.60 (1H, dq, 6.3, 3.6)
3	46.1	2.54 (1H, dq, 13.8, 7.0)	3	44.7	2.67 (1H, dq, 7.2, 3.6)
4	199.9		4	201.5	
4a	104.4		4a	103.9	
5	160.5		5	160.7	
6	110.6		6	110.6	
6a	156.4		6a	156.6	
8	166.7		8	166.7	
9	36.7	3.05 (1H, dd, 15.9, 1.5, Hβ) 2.99 (1H, dd, 15.9, 7.0, Hα)	9	36.6	3.08 (1H, dd, 15.9, 1.5, Hβ) 3.00 (1H, dd, 15.9, 7.1, Hα)
10	34.6	4.56 (1H, d, 5.9)	10	34.5	4.59 (1H, d, 6.7)
10a	104.0		10a	104.1	
10b	156.2		10b	156.1	
11	141.4		11	141.3	
12, 16	126.9	7.12 (2H, d, 7.6)	12, 16	126.8	7.12 (2H, d, 7.6)
13, 15	129.1	7.28 (2H, t, 7.3)	13, 15	129.1	7.28 (2H, t, 7.4)
14	127.5	7.22 (1H, t, 7.3)	14	127.4	7.22 (1H, t, 7.2)
17	19.6	1.42 (3H, d, 6.2)	17	16.1	1.26 (3H, d, 6.6)
18	10.6	1.22 (3H, d, 6.8)	18	9.6	1.09 (3H, d, 7.2)
1′	26.0	3.41(1H, dd, 14.1, 7.2) 3.36 (1H, dd, 14.1, 7.2)	1′	26.0	3.41(1H, dd, 14.3, 7.4) 3.36 (1H, dd, 14.3, 7.1)
2′	121.6	5.23 (1H, t, 6.6)	2′	121.6	5.25 (1H, t, 7.0)
3′	132.7		3′	132.7	
4′	18.1	1.81 (3H, s)	4′	18.1	1.81 (3H, s)
5′	21.5	1.68 (3H, s)	5′	21.5	1.69 (3H, s)
HO-5		12.31 (1H, s)	HO-5		12.23 (1H, s)

[a] The assignments were based on ^{1}H−^{1}H COSY, HMQC, and HMBC experiments.

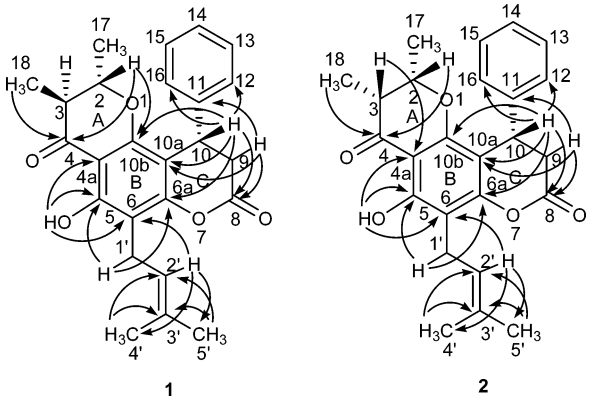

Figure 1. Selected HMBC correlations (→) of **1** and **2**.

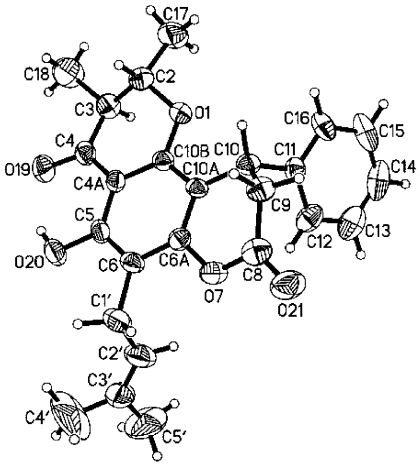

Figure 2. ORTEP drawing of compound **1**.

Compound **2** was also obtained as pale yellow needles. The ion peak at *m*/*z* 405.1714 [M − H]$^-$ in the HRESIMS spectrum gave the molecular formula $C_{25}H_{26}O_5$, which was isomeric with **1**.

Based on H−H COSY, HMQC, and HMBC experiments, structure **2** could be assigned (Figure 1).

The relative configurations at C-2, C-3, and C-10 were determined from the observed $J_{H2,H3}$ (3.6 Hz) and NOESY cross-peak between H-16 and H-17. Accordingly, the structure of compound **2** was defined as 5-hydroxy-2α,3α-dimethyl-6-(3-methylbut-2-enyl)-10α-phenyl-2,3,9,10-tetrahydropyrano[2,3-*f*]chromen-4,8-dione (calopolyanolide D), which is a diastereomers of **1**.

Compounds **1** and **2** did not display any activity against Herpes simplex virus (HSV-2, GI_{50} > 250 μg/mL).

Experimental Section

General Experimental Procedures. Melting points were determined using an XRC-1 melting point apparatus (Sichuan University Science Instruments Factory) and are uncorrected. UV and IR spectra were obtained on a Lambda 35 US/vis spectrometer and a Perkin-Elmer FT-IR spectrometer (KBr disk), respectively. Optical rotations were measured on a Perkin-Elmer 341 automatic polarimeter. Mass spectra were obtained on a Finnigan-LCQ^{DECA} mass spectrometer (ESIMS) and API Q-STAR PULSAR i mass spectrometer (HRESIMS). NMR spectra were recorded on a Bruker Advance 600 MHz spectrometer with TMS as internal standard. X-ray crystallographic data were collected on a Siemens P4 four-circle diffractometer. Silica gel (160−200 and 200−300 mesh, Qingdao Haiyang Chemical Co., Ltd.) was used for column chromatography. RP-18 silica gel was purchased from Phenomenex Co. (Prepex 40−63 μm). Precoated plates (silica gel GF_{254}, 0−40 μm), activated at 110 °C for 2 h, were used for TLC. The visualization of TLC was succeeded by spraying $FeCl_3$−HCl reagent or by UV light (254 and 365 nm). All solvents were distilled prior to use.

Plant Material. The seeds of *C. polyanthum* were collected from Xishuangbanna, Yunnan Province of China, in November 1999. The plant material was identified by Prof. Jing-Yun Cui at Xishangbanna Tropical Botanical Garden, the Chinese Academy of Sciences (CAS). A voucher specimen (GF-129) was deposited at the Herbarium of Chengdu Institute of Biology, CAS.

Extraction and Separation. The air-dried and powdered seeds of *C. polyanthum* (4.5 kg) were soaked with 95% EtOH (25 L × 3, 5 days each) at room temperature. The EtOH was evaporated under reduced pressure to give 690 g of residue, which was suspended in H_2O (1000 mL) and extracted with EtOAc (4 L × 4) and n-BuOH (3.5 L × 4) to give fractions A (350 g) and B (230 g), respectively. Fracton A was subjected to silica gel column chromatography (1300 g, 160−200 mesh, ϕ 80 mm × 470 mm). Gradient elution using petroleum ether (60−90 °C)−acetone (200:1, 100:1, 50:1, 20:1, 5:1, each 6000 mL) yielded five subfractions, A1−A5. Two grams of subfraction A3 (20 g) was chromatographed over silica gel (100 g, 160−200 mesh, ϕ 50 mm × 100 mm) eluted with petroleum ether (60−90 °C)−EtOAc (30:1, 1000 mL) to afford **3** (20 mg), **4** (18 mg), **5** (24 mg), and **6** (40 mg). Subfraction A4 (4 g) was subjected to column chromatography over silica gel (400 g, 200−300 mesh, ϕ 60 mm × 270 mm) and eluted with petroleum ether (60−90 °C)−EtOAc (15:1, 2000 mL) to give **1** (280 mg) and **2** (250 mg). Subfraction A5 (1.5 g, 43 g) was separated on a silica gel column (60 g, 160−200 mesh, ϕ 40 mm × 85 mm) with petroleum ether (60−90 °C)−EtOAc (5:1, 1800 mL) to give **14** (10 mg) and **10** (32 mg). Fraction B (46 g, 230 g) was first subjected to a macroporous resin column (D_{101}, pore size 13−14 nm, 26−60 mesh, ϕ 90 mm × 500 mm) to remove sugars by CH_3OH-H_2O (0:1, 1:0, each 5000 mL), and fraction B′ (6 g) was obtained. Fraction B′ was then chromatographed over silica gel (380 g, 160−200 mesh, ϕ 60 mm × 250 mm) eluting with $CHCl_3-CH_3OH$ (20:1, 10:1, 5:1, 0:1, each 2200 mL) to afford four subfractions, B′1−B′4. Separation of subfraction B′1 (800 mg) by column chromatography (50 g, 200−300 mesh, ϕ 40 mm × 75 mm) with $CHCl_3-CH_3OH$ (20:1, 1000 mL) afforded **13** (15 mg). Subfraction B′2 (1.1 g) was chromatographed over silica gel (50 g, 200−300 mesh, ϕ 40 mm × 75 mm) eluting with $CHCl_3-CH_3OH$ (10:1, 1200 mL) to give **12** (20 mg) and **11** (24 mg). **7** (50 mg) and **8** (42 mg) were obtained from subfraction B′3 (2.2 g) by silica gel column chromatography (100 g, 200−300 mesh, ϕ 40 mm × 150 mm) with $CHCl_3-CH_3OH$ (5:1, 3000 mL). Subfraction B′4 (900 mg) was chromatographed by a reverse-phase R-18 silica gel column (35 g, 40−63 μm, ϕ 30 mm × 95 mm) eluted with CH_3OH-H_2O (2:3, 500 mL) to yield **9** (90 mg).

Calopolyanolide C (1): pale yellow needles ($CHCl_3$); mp 127−128 °C; $[\alpha]_D^{20}$ −193.2° (*c* 0.132, $CHCl_3$); TLC, R_f 0.40 (petroleum ether (60−90 °C)−EtOAc, 10:1); UV (CH_3OH) λ_{max} nm (log ϵ) 348 (3.56), 284 (4.18), 221 (4.33); IR ν_{max} cm^{-1} 3435, 2926, 1780, 1630, 1605, 1494, 1454, 1167, 1124, 1103; ^1H NMR ($CDCl_3$, 600 MHz) and ^{13}C NMR (150 MHz) data, see Table 1; ESIMS (negative mode) *m*/*z* 405 $[M - H]^-$; HRESIMS (negative mode) *m*/*z* 405.1715 ($[M - H]^-$ calcd for $C_{25}H_{25}O_5$, 405.1702).

Calopolyanolide D (2): pale yellow needles ($CHCl_3$); mp 157−158 °C; $[\alpha]_D^{20}$ −34.1° (*c* 0.132, $CHCl_3$); TLC, Rf 0.45 (petroleum ether (60−90 °C)−EtOAc, 10:1); UV (CH_3OH) λ_{max} nm (log ϵ) 352 (3.55), 285 (4.18), 229 (4.32); IR ν_{max} cm^{-1} 3432, 2922, 1781, 1642, 1605, 1495, 1448, 1170, 1132, 1098; ^1H NMR ($CDCl_3$, 600 MHz) and ^{13}C NMR (150 MHz) data, see Table 1; ESIMS (negative mode) *m*/*z* 405 $[M - H]^-$; HRESIMS (negative mode) *m*/*z* 405.1714 ($[M - H]^-$ calcd for $C_{25}H_{25}O_5$, 405.1702).

Calopolyanolide A (3): pale yellow oil; $[\alpha]_D^{20}$ −170.2° (*c* 0.213, $CHCl_3$); TLC, R_f 0.30 (petroleum ether (60−90 °C)−acetone, 10:1); UV, IR, optical rotation, and ^1H NMR data are consistent with literature values;[15] ESIMS (negative mode) *m*/*z* 421 $[M - H]^-$.

Calopolyanolide B (4): pale yellow oil; $[\alpha]_D^{20}$ −105.5° (*c* 0.241, $CHCl_3$); TLC, R_f 0.27 (petroleum ether (60−90 °C)−acetone, 10:1); UV, IR, and ^1H NMR data are consistent with literature values;[15] ESIMS (negative mode) *m*/*z* 421 $[M - H]^-$.

Calanolide E2 (5): pale yellow oil; $[\alpha]_D^{20}$ −77.6° (*c* 0.248, $CHCl_3$); TLC, R_f 0.20 (petroleum ether (60−90 °C)−acetone, 10:1); UV, IR, optical rotation, ^1H NMR, and ^{13}C NMR data are consistent with literature values;[17] ESIMS (negative mode) *m*/*z* 387 $[M - H]^-$.

5,7,3′,4′-Tetrahydroxyisoflavone (6): pale yellow powder; mp 267−268 °C (lit.[18] 270 °C); TLC, R_f 0.56 (petroleum ether (60−90 °C)−EtOAc, 1:1); IR ν_{max} cm^{-1} 3374, 2919, 2850, 1666, 1633, 1588, 1496, 1450, 1346, 1279, 1044, 880, 761; UV data are consistent with literature values;[19] ESIMS (negative mode) *m*/*z* 285 $[M - H]^-$.

Compounds **7**−**14** were identified by comparison of melting points and TLC behavior with those of authentic compounds.

X-ray Crystal Data of 1.[20] A pale yellow crystal was obtained from acetone−H_2O (1:5). Crystal data: $C_{25}H_{26}O_5$; M_r = 406.46; dimensions 0.60 × 0.50 × 0.20 mm; orthorhombic, space group $P2_12_12_1$, a = 10.044(2) Å, b = 10.216(2) Å, c = 21.784(4) Å, $\alpha = \beta = \gamma = 90°$, V = 2235.3(5) Å^3, Z = 4, D_{calc} = 1.208 g/cm^3, λ = 0.71073 Å, μ(Mo Kα) = 0.083 mm^{-1}, $F(000)$ = 864, T = 290(2) K. Of the 3050 reflections that were collected, 2771 were unique (R_{int} = 0.0143). The structure was solved by direct methods with SHELXS97[21] and refined by full-matrix least-squares on F^2. Final refinement: data/restraints/parameters = 2771/0/280; R_1 = 0.1146 (all data), wR_2 = 0.0700 (all data). Absolute structure parameter = 0(10), and GOF = 0.801. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.112 and −0.115 e$^-$/Å^3, respectively.

Antiherpetic Activity Assay. Inhibition of Herpes simplex virus type 2 (HSV-2) was evaluated in the Vero cell line by assaying the appearance of cytopathic effect (CPE), after the tested compound was applied at various concentrations.[22] Acyclovir (ACV) was used as positive drug control.

Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

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(20) Crystallographic data (including structure factors) for compound **1** reported in this paper have been deposited with the Cambridge Crystallographic Data Center (deposition number CCDC230762). Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK [fax: +44-0-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].
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