JOURNAL OF NATURAL PRODUCTS

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Bioactive Diterpenes from *Callicarpa longissima*

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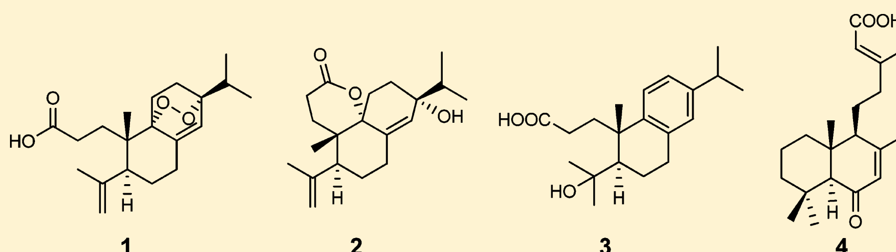
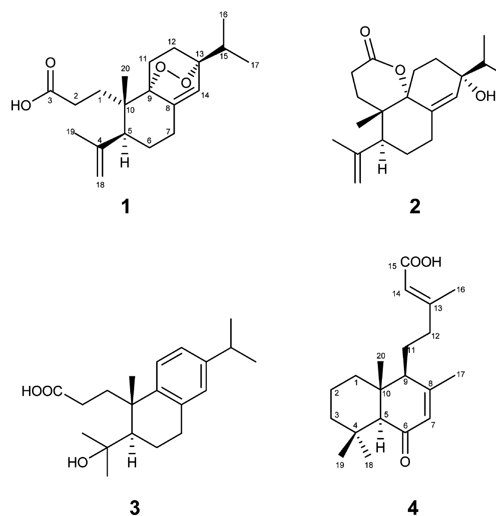
Supporting Information



ABSTRACT: Investigation of the leaves and twigs of *Callicarpa longissima* resulted in the isolation of four new compounds (**1**–**4**), callilongisins A–D, and five known compounds, ursolic acid, 3-oxoanticopalic acid, (*E*)-6*β*-hydroxylabda-8(17),13-dien-15-oic acid, 5-hydroxy-3,6,7,4′-tetramethoxyflavone, and artemetin. Compounds **1**–**3** are 3,4-*seco*-abietane-type diterpenoids, and compound **4** is an analogue of a labdenoic-type diterpene. The structure of compound **1** was confirmed by X-ray crystallographic analysis. Cytotoxicity against a human prostate cancer cell line (PC3) and anti-inflammatory activities of the isolated compounds were evaluated.

Beautyberry, *Callicarpa longissima* (Hemsl.) Merr. (Verbenaceae), is a shrub that grows in mountainous areas of Taiwan and mainland China. The genus *Callicarpa* comprises 190 species, and 12 species have been found in Taiwan. Reported components include terpenoids,[1−3] lignans,[4] glycosides,[5] and flavonoids,[6] and some of them were tested to show cytotoxic,[7−9] mosquito repellent,[10] antibacterial,[11] antivial,[12] and anthelmintic[13]activities. In traditional Chinese medicine, preparations of beautyberry have been used for the treatment of the common cold, cough, arthritis, bleeding, and abdominal pain.[14] In a recent study of a close species, *C. pilosissima*, *seco*-abietane diterpenoids and an *α*-tocopherol trimer were reported to have antitubercular activity.[15] Herein, we report the isolation, and structural elucidation of four new natural products, callilongisins A–D (**1**–**4**), along with ursolic acid (**5**),[16] 3-oxoanticopalic acid (**6**),[17] (*E*)-6*β*-hydroxylabda-8-(17),13-dien-15-oic acid (**7**),[18] 5-hydroxy-3,6,7,4′-tetramethoxyflavone (**8**),[19] and artemetin (**9**)[20] from an ethanolic extract of twigs and leaves of *C. longissima*. The structures of all compounds were established by interpretation of their spectroscopic data, especially 2D NMR. The relative configurations of **1**–**4** were unambiguously assigned by NOESY experiments, and the structure of **1** was confirmed by X-ray crystallographic analysis. Selected compounds were also evaluated for anti-inflammatory and for cytotoxic activity against a human prostate cancer cell line.



RESULTS AND DISCUSSION

Callilongisin A (**1**) was obtained as yellowish prisms and had the molecular formula $C_{20}H_{30}O_4$ (Δ = 6), as deduced from HRESIMS (*m*/*z* 357.2044 $[M + Na]^+$) and DEPT NMR spectra. The IR spectrum showed the presence of OH (3445 cm^{-1}), acid carbonyl (1705 cm^{-1}), and C=C double-bond (1645 cm^{-1}) groups. The ^1H NMR data of **1** (Table 1)

Received: November 29, 2011
Published: March 19, 2012

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Table 1. ^{1}H NMR Spectroscopic Data for 1−4[a]

position	1	2	3	4
1	1.75 m	1.69 m	2.07 m	1.20 m
	1.93 m	1.89 m	2.83 m	1.86 d (12.8)
2	2.55 m	2.67 m	1.91 m	1.50 m
			2.18 m	1.60 m
3				1.15 m
				1.36 m
5	2.63 m	2.59 t (10.6)	1.70 m	2.06 s
6	1.73 m	1.70 m	1.60 m	
		1.83 m	1.73 m	
7	2.56 m	2.20 m	2.71 m	5.78 s
		2.46 m		
9				2.09 s
11	1.38 m	1.80 m	7.20 d (8.4)	1.57 m
	2.20 m	2.38 m		1.64 m
12	1.48 m	1.54 m	7.00 d (8.4)	2.21 m
	1.96 m			2.43 m
14	6.10 s	5.64 s	6.83 s	5.73 s
15	1.90 m	1.72 m	2.83 m	
16	0.98 d (6.5)	0.95 d (6.8)	1.22 d (6.8)	2.21 s
17	0.98 d (6.5)	0.91 d (6.8)	1.22 d (6.8)	1.94 s
18	4.88 s	4.79 s	1.34 s	1.13 s
	4.88 s	4.98 s		
19	1.81s	1.80 s	1.30 s	1.16 s
20	1.05 s	1.01 s	1.47 s	0.85 s

[a]Chemical shifts (δ) are in ppm; *J* values in Hz are in parentheses.

exhibited signals of two methyl singlets (δ 1.05 and 1.81), two methyl doublets (δ 0.98), an olefinic methine singlet (δ 6.10), and an exocyclic methylene singlet (δ 4.88). The ^{13}C NMR (Table 2) and HSQC spectra of **1** showed 20 carbon signals, consisting of one acid carbonyl carbon (δ 180.3), two double bonds (δ 114.8, 126.7, 145.0, 147.3), two oxygenated quaternary carbons (δ 79.3, 82.8), an aliphatic quaternary carbon (δ 41.4), two aliphatic methines (δ 32.3, 46.3), six aliphatic methylenes (δ 23.0, 24.1, 24.7, 25.4, 30.1, 30.8), and four methyl carbons (δ 17.0, 17.4, 17.6, 23.9). In the COSY spectrum of **1**, correlations from three pairs of adjoining methylenes, H-1 (δ 1.73)/H-2 (δ 2.55), H-6 (δ 1.73)/H-7 (δ 2.56), and H-11 (δ 2.20)/H-12 (δ 1.96), as well as an isopropyl moiety, H-16 (δ 0.98)/H-15 (δ 1.90)/H-17 (δ 0.98), were observed. The isopropyl group was attached to C-13, as evidenced by the HMBC correlation of H-17/C-13 (δ 79.3) (Figure 1). In addition, a pair of correlations, H-1/C-10 (δ 41.4) and H-2/C-3 (δ 180.3), indicated the presence of a propionic acid moiety substituted at C-10. An isopropenyl group attached at C-5 was revealed by the correlations of H-19 (δ 1.81)/C-4 (δ 147.3), C-5 (δ 46.3), and C-18 (δ 114.8). HMBC correlations of H-20 (δ 1.05)/C-1 (δ 30.1), C-5, C-9 (δ 82.8), and C-10; H-7 (δ 2.56)/C-8 (δ 145.0), C-9, and C-14 (δ 126.7); and H-14 (δ 6.10)/C-9 and C-13 could construct a bicyclic ring system with a methyl group at C-10. Thus the above 2D NMR revealed that **1** was a 3,4-*seco*-abietane-type diterpene.[6] The presence of two C═C bonds, an acid group, and a bicyclic ring system accounted for five of the six degrees of unsaturation and two of the four oxygen atoms; the remaining two oxygen atoms could only be accounted for by a peroxide bridge between two quaternary carbons (C-9 and C-13). Thus, the planar structure of **1** was established.

Table 2. ^{13}C NMR Spectroscopic Data (δ) for 1−4[a]

position	1	2	3	4
1	30.1 CH_2	26.9 CH_2	36.4 CH_2	39.0 CH_2
2	30.8 CH_2	26.3 CH_2	29.6 CH_2	18.3 CH_2
3	180.3 C	172.0 C	180.3 C	43.2 CH_2
4	147.3 C	145.6 C	74.9 C	32.5 C
5	46.3 CH	44.2 CH	48.8 CH	63.7 CH
6	24.1 CH_2	26.8 CH_2	24.3 CH_2	200.1 C
7	24.7 CH_2	31.5 CH_2	31.7 CH_2	129.1 CH
8	145.0 C	137.1 C	137.5 C	157.7 C
9	82.8 C	85.6 C	142.0 C	55.9 CH
10	41.4 C	38.4 C	42.9 C	43.4 C
11	23.0 CH_2	27.1 CH_2	126.4 CH	25.3 CH_2
12	25.4 CH_2	26.4 CH_2	124.3 CH	43.5 CH_2
13	79.3 C	71.3 C	145.5 C	162.2 C
14	126.7 CH	131.1 CH	126.3 CH	115.6 CH
15	32.3 CH	38.0 CH	33.3 CH	170.5 C
16	17.4 CH_3	16.4 CH_3	23.8 CH_3	19.4 CH_3
17	17.6 CH_3	17.2 CH_3	23.8 CH_3	22.2 CH_3
18	114.8 CH_2	115.2 CH_2	33.3 CH_3	33.6 CH_3
19	23.9 CH_3	22.6 CH_3	27.0 CH_3	21.7 CH_3
20	17.0 CH_3	18.8 CH_3	27.5 CH_3	14.9 CH_3

[a]Assignments were made using HMQC and HMBC techniques.

The relative configuration of **1** was determined on the basis of NOESY correlations (Figure 2). On the basis of biogenetic studies of 3,4-*seco*-abietane derivatives,[3] the CH_3-20 group was considered to be β-oriented. The NOESY spectrum of **1** showed correlations of H-20/H-18/H-19, indicating that they are on the β-face of the molecule. On the contrary, the presence of a NOESY cross-peak between H-1/H-5 suggested that they were α-oriented. In order to determine the configurations of all other chiral centers, **1** was crystallized from a mixture of ethanol and methylene chloride (2:1). An X-ray crystallographic analysis of the prism (Figure 3) confirmed the relative configurations deduced by NOESY experiment and also revealed that its peroxide bridge was located on the α-face. The above observations established the structure of callilongisin A (**1**) unambiguously.

Callilongisin B (**2**) had the molecular formula $C_{20}H_{30}O_3$, as deduced from HRESIMS (*m/z* 341.2081 [M + Na]$^+$) and DEPT spectra. The IR absorption bands revealed the presence of OH (3466 cm^{-1}) and carbonyl (1711 cm^{-1}) groups in **2**. The 1D and 2D NMR spectra of **2** were similar to those of **1**, suggesting that it was a close analogue. On comparing the ^{13}C NMR spectra of **1** and **2**, it was noted that the C-3 chemical shift of **2** (δ 172.0) was upfield compared to that of **1** (δ 180.3), and the shift value of C-9 (δ 85.6) was downfield compared to that of the same carbon in **1** (δ 82.8). Thus it was suggested that C-3 was linked with C-9 to form a lactone ring in **2**. This was confirmed by the HMBC J_4 correlation from H-11 (δ 1.80) to C-3. The combined COSY and HMBC correlations showed that all other structural fragments were similar to those of **1**, confirming that compound **2** is a 3,9-lactonide derivative of **1**, in which the peroxide bridge was absent and had been replaced with OH and isopropyl groups attached to C13.

The relative configuration of **2** was determined by NOESY experiment (Figure 2), in which the CH_3-20 group was assigned to be β-oriented on the basis of the known biogenetic pathway of related diterpenoids. NOESY correlations between H-20/H-18 (δ 4.79), H-19 (δ 1.80), H-7β (δ 2.46), and H-15 (δ 1.72) indicated that the isopropenyl group at C-5 and the isopropyl at C-13 were both β-oriented. On the contrary, an α-

dx.doi.org/10.1021/np200932k | J. Nat. Prod. 2012, 75, 689−693

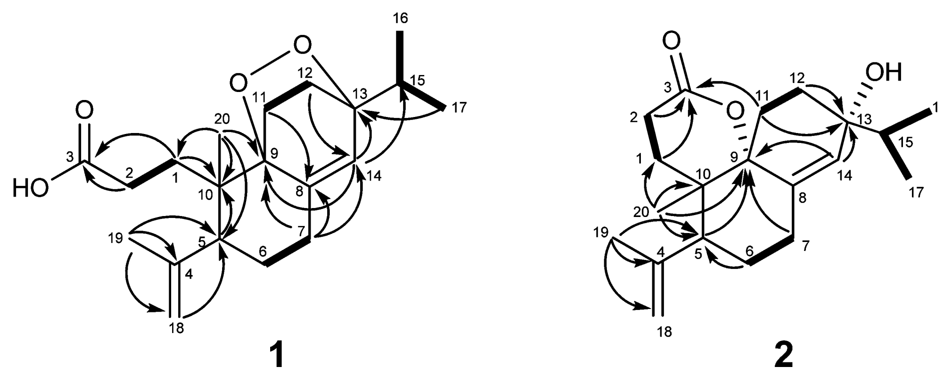

Figure 1. Key COSY (bold bonds) and HMBC correlations (arrows) of **1** and **2**.

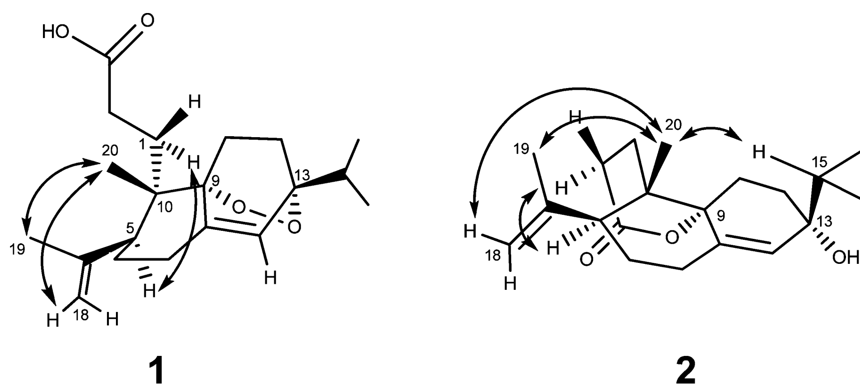

Figure 2. Key NOESY correlations of **1** and **2**.

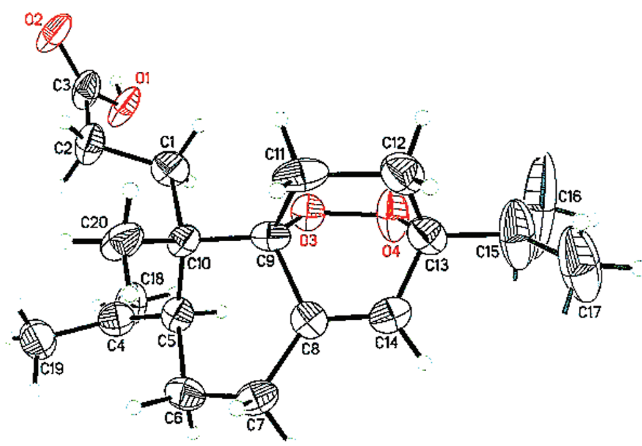

Figure 3. ORTEP drawing of the X-ray structure of **1**.

oriented δ-lactone ring was revealed by the NOESY cross-peak between H-5 (δ 2.59)/H-2 (δ 2.67). Therefore, the structure of callilongisin B (**2**) was established.

Callilongisin C (**3**) had the molecular formula $C_{20}H_{30}O_3$, as deduced from HRESIMS (*m/z* 341.2068 $[M + Na]^+$). The UV spectrum of **3** showed absorption bands at 251 and 275 nm, which implied the presence of a benzene moiety. The ^1H and ^{13}C NMR spectra of **3** revealed a substitution pattern consisting of a methyl (C-20), a propionic acid (C-1 to C-3), and an isopropyl (C-15 to C-17) group, similar to that of **1**. However, the HMBC correlations from two geminal methyl protons (δ 1.30 and 1.34) to C-4 (δ 74.9) and C-5 (δ 48.8) indicated that a hydroxyisopropyl group at C-5 was different from the isopropenyl substitution in **1**. Moreover, HMBC correlations of H-11 (δ 7.20)/C-8 (δ 137.5), C-12 (δ 124.3), and C-13 (δ 145.5) and of H-12 (δ 7.00)/C-9 (δ 142.0) and C-14 (δ 126.3) indicated that the cyclohexene ring in **1** was replaced with a benzene ring in **3**. Indeed, a pair of *ortho* couplings (*J* = 8.4 Hz) in the aromatic ring moiety were observed. The relative configuration was determined by the NOESY experiment, based upon the assumption of a β-oriented CH_3-20 group. The geminal methyl protons CH_3-18 and CH_3-19 showed NOE correlations with H-5, suggesting that the dimethylcarbinol group was β-oriented. Thus the structure of **3** was established, and it was named callilongisin C.

Callilongisin D (**4**), $[\alpha]_D^{25}$ 1.56 ($CHCl_3$), had the molecular formula $C_{20}H_{30}O_3$ (Δ = 6), as established from its HRESIMS (*m/z* 341.2090 $[M + Na]^+$) and DEPT spectra. The UV spectrum showed bands at 246 and 329 nm, indicating the presence of a conjugated ketone chromophore. The similarity of the NMR data of **4** and (*E*)-6β-hydroxylabda-8(17),13-dien-15-oic acid (**7**) suggested that these compounds were close analogues. HMBC correlations of H-7 (δ 5.78)/C-6 (δ 200.1) and of H-17 (δ 1.94)/C-7 (δ 129.1) and C-8 (δ 157.7) indicated that the C-6 carbonyl group was conjugated with a C-7, C-8 double bond. The double bond between C-13 and C-14 was assigned *E*-geometry as a result of a NOESY correlation between H-14 (δ 5.73) and H-12 (δ 2.43). NOESY correlations between H-20 (δ 0.85)/H-19 (δ 1.16) and H-20/H-11 (δ 1.57) indicated that these protons were β-oriented. On the contrary, NOESY cross-peaks between H-18 (δ 1.13)/H-5 (δ 2.06)/H-9 (δ 2.09) suggested that they were on the α-face. The above observations were used to establish the structure of **4** as shown, and it was named callilongisin D.

The isolation of new diterpenes (**1**−**4**) from *C. longissima* may be significant from the viewpoint of chemotaxonomy. These compounds are useful as biological markers in differentiating species of *Callicarpa*. A biogenetic pathway to

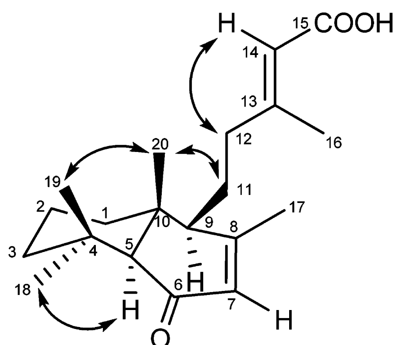

Figure 4. Key NOESY correlations of **4**.

compounds **1**−**3** was proposed as illustrated in Scheme 1, Supporting Information. The compounds were considered to be produced from the common precusor geranyl geranyl pyrophosphate through cyclization to give (−)-sandaracopimaradiene, followed by Wagner−Meerwein 1,2 methyl shift, hydroxylation, and oxidation to yield intermediate a, which could produce intermediates b and c via Baeyer−Villiger oxidation and hydrolysis. Subsequent hydroxylation and dehydration of intermediate c may afford compounds **1**−**3** or, via other steps, yield 12-deoxy-11,12-dihydro-*seco*-hinokiol, respectively.[15]

The isolated compounds were tested for their in vitro inhibitory effects against human prostate cancer cell line (PC-3) and for their effects on superoxide anion generation and elastase release by human neutrophils in response to FMLP/CB. Compounds **1** and **4**−**6** showed significant anti-inflammatory effects (Table 3). None of these compounds showed significant cytotoxicity against PC-3 (IC_{50} > 10 μg/mL).

Table 3. Effects of Compounds 1−9 on Superoxide Anion Generation and Elastase Release by Human Neutrophils in Response to FMLP/CB[a]

	superoxide anion	elastase release
compound	IC_{50} (μg/mL)	IC_{50} (μg/mL)
1	3.14	4.66
4	5.09	>10
5	2.79	5.21
6	9.31	>10

[a]The IC_{50} values of compounds **2**, **3**, and **7**−**9** are larger than 10 μg/mL.

■ EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were recorded on a JASCO DIP-1000 polarimeter. IR spectra were taken on a Horibaft-720 spectrophotometer. The ^{1}H and ^{13}C NMR spectra as well as 2D NMR spectra (COSY, HMQC, HMBC, and NOESY) were recorded in $CDCl_3$ on a Bruker AVX NMR spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C using the $CDCl_3$ solvent peak as internal standard (δ_H 7.26, δ_C 77.0 ppm). Low-resolution EIMS spectra were recorded on a VG Quattro 5022 mass spectrometer. HRESIMS were measured on a JEOL HX 110 mass spectrometer. LiChrospher Si 60 (5 μm, 250-10, Merck) and LiChrospher 100 RP-18e (5 μm, 250-10, Merck) were used for NP-HPLC and RP-HPLC (Hitachi, L-6250; flow rate 2 mL/min, UV detection at 254 nm), respectively.

Collection, Identification, and Extraction. The twigs and leaves (420 g) of *C. longissima* were collected in Shin-Chu County, Taiwan, in July 2009. The plant material was identified by one of the authors (Y.C.S.). A voucher specimen (code no. CP0710) has been deposited in the School of Pharmacy, College of Medicine, National Taiwan University, Taipei, Taiwan.

Isolation of Compounds 1−9. The plant material was extracted three times with ethanol to afford a crude extract (75 g). This was partitioned between EtOAc and H_2O (1:1) to obtain an EtOAc-soluble layer, which afforded an EtOAc extract (25.3 g) after evaporation. This extract was repartitioned between *n*-hexane−MeOH−H_2O (4:3:1) to give a MeOH-soluble residue (10.4 g). Then, the residue was chromatographed on a Si gel column using gradient elution of *n*-hexane−EtOAc−MeOH (10:1:0 to 0:0:1) to furnish fractions 1−10. Fraction 6 (0.9 g) was separated by a Si gel column (*n*-hexane−EtOAc−MeOH, 3:1:0 to 0:0:1) to furnish subfractions 6A−6G. Subfraction 6E (54 mg) was subjected to RP-HPLC (C_{18}, 10 mm × 250 mm, MeOH−H_2O−CH_2Cl_2, 5:2:1, plus 1% formic acid), which afforded (*E*)-6β-hydroxylabda-8(17),13-dien-15-oic acid (**7**, 6.3 mg) and 5-hydroxy-3,6,7,4′-tetramethoxyflavone (**8**, 2.6 mg). Subfraction 6F (280 mg) was dissolved in MeOH to give an insoluble ursolic acid (**5**, 30 mg) and a soluble fraction. The latter fraction was subjected to RP-HPLC (C_{18}, 10 mm × 250 mm, MeOH−H_2O−CH_2Cl_2, 7:3:1) to afford **4** (6 mg) and 3-oxoanticopalic acid (**6**, 3 mg). Subfraction 6G (560 mg) was separated on a Si gel column (*n*-hexane−EtOAc−MeOH, 10:1:0 to 0:0:1) to give **1** (32 mg). Fraction 7 (3.36 g) was further purified by a Sephadex LH-20 column (MeOH−CH_2Cl_2, 1:1) to yield **5** (48 mg) and subfractions 7A−7E. Separation of subfraction 7D (1.3 g) on a Si gel column (*n*-hexane−acetone−CH_2Cl_2, 3:1:2) yielded subfraction 7D1 (18.6 mg) and **9** (200 mg). Subfraction 7D1 was further purified by RP-HPLC (C_{18}, 10 mm × 250 mm, MeOH−H_2O−CH_2Cl_2, 6:3:1) to yield **2** (6 mg). Subfraction 7E (1.8 g) was subjected to a Si gel column (*n*-hexane−EtOAc−CH_2Cl_2, 6:4:1) to furnish fraction 7E9 (130 mg). The latter fraction was chromatographed on a Si gel column (*n*-hexane−CH_2Cl_2−MeOH, 6:3:1) to afford **3** (16 mg).

*Callilongisin A (**1**):* yellowish prisms; $[\alpha]^{25}_{D}$ 4.4 (*c* 1.0, MeOH); mp 130 °C; IR (neat) ν_{max} 3445, 2933, 1705, 1645, 1417, 1385, 1298, 1215, 1179 cm^{-1}; UV λ_{max} (log ε) 230 (3.26); ^{1}H NMR and ^{13}C NMR spectroscopic data, see Tables 1 and 2, respectively; HRESIMS *m/z* 357.2044 ([M + Na]$^+$, calcd for $C_{20}H_{30}O_4Na$, 357.2042).

Crystal data of **1**: $C_{20}H_{30}O_4$, formula weight 334.4498, orthorhombic system, space group *P*21, *a* = 12.148(5) Å, *b* = 6.279(13) Å, *c* = 12.403(8) Å, *V* = 948.3(8) Å^3, *Z* = 2, *d* = 1.177 Mg/m^3. A crystal of dimensions 0.20 × 0.15 × 0.10 mm was used for measuring on a Siemens SMART CCD XRD. The total number of independent reflections measured was 2992, of which 2336 were observed [*R*(int) = 0.0464]. Completeness to θ = 67.99°: 99.1%, absorption correction: semiempirical from equivalents. Max. and min. transmission: 1.000 00 and 0.659 58. The structure was solved by direct methods and refined by a full-matrix least-squares on F^2. Final *R* indices [$I > 2\sigma(I)$]: R1 = 0.1081, wR2 = 0.3286. The final X-ray diagram is shown in Figure 3. Crystallographic data for **1** reported in this paper have been deposited with the Cambridge Crystallographic Data Center (CCDC 863566). Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

*Callilongisin B (**2**):* yellowish oil; $[\alpha]^{25}_{D}$ 6.0 (*c* 0.1, MeOH); IR (neat) 3466, 2928, 1711, 1463, 1382, 1267, 1038, 972 cm^{-1}; UV λ_{max} (log ε) 218 (3.10); ^{1}H NMR and ^{13}C NMR spectroscopic data, see Tables 1 and 2, respectively; HRESIMS *m/z* 341.2081 ([M + Na]$^+$, calcd for $C_{20}H_{30}O_3Na$, 341.2093).

*Callilongisin C (**3**):* yellowish oil; $[\alpha]^{25}_{D}$ 7.8 (*c* 0.1, MeOH); IR (neat) 3413, 2961, 1708, 1497, 1461, 1378, 1192 cm^{-1}; UV λ_{max} (log ε) 251 (3.22), 275 (2.89); ^{1}H NMR and ^{13}CNMR spectroscopic data, see Tables 1 and 2, respectively; HRESIMS *m/z* 341.2068 ([M + Na]$^+$, calcd for $C_{20}H_{30}O_3Na$, 341.2093).

dx.doi.org/10.1021/np200932k | *J. Nat. Prod.* 2012, 75, 689−693

Callilongisin D (4): yellowish oil; $[\alpha]^{25}_{D}$ 1.6 (*c* 0.1, $CHCl_3$); IR (neat) 2927, 1688, 1643, 1460, 1358, 1256, 1163 cm^{-1}; UV λ_{max} (log ε) 246 (3.17), 329 (2.75); ^1H NMR and ^{13}C NMR spectroscopic data, see Tables 1 and 2, respectively; HRESIMS *m/z* 341.2090 ($[M + Na]^+$, calcd for $C_{20}H_{30}O_3Na$, 341.2093).

Cytotoxicity Assay. The bioassay against PC-3 (human androgen-independent prostate carcinoma) tumor cells was based on the sulforhodamine B assay method. Cells were inoculated into 96-well microtiter plates in RPMI 1640 medium containing 5% fetal bovine serum and incubated at 37 °C, 5% CO_2, 95% air. After 24 h, two plates of each cell line were fixed in situ with trichloroacetic acid (TCA). Following compound addition, the plates were incubated for an additional 48 h at 37 °C, 5% CO_2, 95% air, and 100% relative humidity. Assays were terminated by the addition of cold TCA. The supernatant was discarded, and the plates were washed five times with tap water, then air-dried. Sulforhodamine B solution (100 μL) at 0.4% w/v in 1% acetic acid was added to each well, and plates were incubated for 10 min at room temperature. After staining, unbound dye was removed by washing three times with 1% acetic acid and the plates were air-dried. Bound stain was subsequently solubilized with 10 mM Trizma base, and the absorbance was read on an automated plate reader at a wavelength of 515 nm. The IC_{50} value was defined, by a comparison with the untreated cells, as the concentration of test sample resulting in a 50% reduction of absorbance.

Anti-inflammatory Assay. Inhibitory Effect on Superoxide Anion Generation and Elastase Release by Human Neutrophils. Neutrophils were obtained by means of dextran sedimentation and Ficoll centrifugation. Superoxide generation and elastase release were carried out according to a procedure described previously.[21] Superoxide anion production was assayed by monitoring the superoxide dismutase-inhibitable reduction of ferricytochrome *c*. Elastase release experiments were performed using MeO-Suc-Ala-Ala-Pro-Valp-nitroanilide as the elastase substrate.

■ ASSOCIATED CONTENT

Supporting Information

NMR spectra of compounds **1**−**4**, structures of the known compounds **5**−**9**, Scheme 1 (proposed biogenetic scheme), X-ray CIF, and crystal data of **1**. This information is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors thank the National Science Council, Republic of China (NSC98-2320-B002-027-MY3), for financial support.

■ REFERENCES

(1) Xu, J.; Harrison, L. J.; Vittal, J. J.; Xu, Y. J.; Goh, S. H. *J. Nat. Prod.* **2000**, *63*, 1062−1065.
(2) Subramanian, S. S.; Nair, A. G. R.; Vedantham, T. N. C. *Phytochemistry* **1974**, *13*, 306−307.
(3) Liu, H. Y.; He, H. P.; Gao, S.; Chen, C. X.; Shen, Y. M.; Hao, X. J. *Helv. Chim. Acta* **2006**, *89*, 1017−1022.
(4) Shao, Y.; Hu, L. H.; Sim, K. Y.; Goh, S. K. *Helv. Chim. Acta* **2006**, *89*, 64−72.
(5) Mei, W. L.; Han, Z.; Cui, H. B.; Zhao, Y. X.; Deng, Y. Y.; Dai, H. F. *Nat. Prod. Res.* **2010**, *24*, 899−904.
(6) Hu, Y. M.; Shen, Y. M.; Gu, Q. X.; Zuo, G. Y.; Hao, X. J. *Chin. Tradit. Herb. Drugs* **2001**, *32*, 1063−1065.
(7) Jones, W. P.; Kinghorn, A. D. *Curr. Bioact. Compd.* **2008**, *4*, 15−32.
(8) Jones, W. P.; Tatiana, L. E.; Qiuwen, M.; Chai, H. B.; Soejarto, D. D.; Cordell, G. A.; Swanson, S. M.; Kinghorn, A. D. *J. Nat. Prod.* **2007**, *70*, 372−377.
(9) Mei, W. L.; Han, Z.; Chi, H. B.; Zhao, Y. X.; Deng, Y. Y.; Dai, H. F. *Nat. Prod. Res.* **2009**, *23*, 1−6.
(10) Cantrell, C. L.; Klun, J. A.; Bryson, C. T.; Kobaisy, M.; Duke, S. O. *J. Agric. Food Chem.* **2005**, *53*, 5948−5953.
(11) Kim, Y. S.; Shin, D. H. *J. Agric. Food Chem.* **2004**, *52*, 781−787.
(12) Woo, E. R.; Yoon, S. H.; Kwak, J. H.; Kim, H. J.; Park, H. *Phytomedicine* **1997**, *4*, 53−58.
(13) Lee, K. Y.; Jeong, E. J.; Lee, H. S.; Kim, Y. C. *Biol. Pharm. Bull.* **2006**, *29*, 71−74.
(14) *The Illustration of Common Medicinal Plants in Taiwan III*; Committee on Chinese Medicine Pharmacy: Department of Health, R.O.C., 2004; p 40.
(15) Chen, J. J.; Wu, H. M.; Peng, C. F.; Chen, I. S.; Chu, S. D. *J. Nat. Prod.* **2009**, *72*, 223−228.
(16) Tkachev, A. V.; Denisov, A. Y.; Gatilov, Y. V.; Bagryanskaya, I. Y.; Shevtsov, S. A.; Rybalova, T. V. *Tetrahedron* **1994**, *50*, 11459−11488.
(17) Braun, S.; Breitenbach, H. *Tetrahedron* **1977**, *33*, 145−150.
(18) Zdero, C.; Bohlmann, F.; King, R. M. *Phytochemistry* **1992**, *31*, 1631−1638.
(19) Sachdev, K.; Kulshreshtha, D. K. *Phytochemistry* **1983**, *22*, 1253−1256.
(20) Ahmad, V. U.; Khan, M. A.; Baqai, F. T.; Tareen, R. B. *Phytochemistry* **1995**, *38*, 1305−1307.
(21) Liaw, C. C.; Shen, Y. C.; Hwang, T. L.; Kuo, Y. H.; Khalil, A. T. *J. Nat. Prod.* **2008**, *71*, 1551−1556.