Two New Abietane Diterpenoids from the Stems of *Clerodendrum kaichianum* P. S. HSU

by **Mingfeng Xu**, **Lianqing Shen***, **Kuiwu Wang**, and **Qizhen Du**

College of Food Science and Biotechnology Engineering, Zhejiang Gongshang University, Hangzhou 310035, P. R. China
(phone: +86-571-88071024-7575; fax: +86-571-88071024-7575; e-mail: zjgsu423@yahoo.cn)

Two new abietane diterpenoids, named 17-hydroxyteuvincenone G (**1**) and 17-hydroxyteuvincen-5(6)-enone G (**2**), together with four known diterpenoids, were isolated from the stems of *Clerodendrum kaichianum* P. S. HSU. Their structures were elucidated by extensive NMR and MS analyses, and by comparison with literature data. The new compounds showed significant cytotoxicities against the HL-60 and A-549 tumor cell lines.

Introduction. – The genus *Clerodendrum* contains more than 30 species distributed in China, some of which have been used in Traditional Chinese Medicine, such as *Clerodendrum indicum* for treating malaria and rheumatism [1]. Plants of the genus *Clerodendrum* have proved to be a rich source of abietane diterpenoids, as well as iridoids, triterpenes, phenylethanoid glycosides, and saponins, which have been found to possess many beneficial pharmacological effects, such as antimalarial, antitumor, and anti-HIV activities [2–8]. In China, the leaves of *Clerodendrum kaichianum* P. S. HSU are used as a traditional medicine against hypertension. Search for the new bioactive natural products from this plant has led to the isolation of two new abietane diterpenoids, **1** and **2**, together with four known abietane diterpenoids, teuvincenone A (**3**), 11,14-dihydroxyabieta-8,11,13-trien-7-one (**4**), dehydroabietan-7-one (**5**), and sugiol (**6**), and these two new compounds were found to show cytotoxicity against HL-60 and A-549 cells. Here, we describe the isolation, structure elucidation, and cytotoxic activity of the new compounds.

Results and Discussion. – The 75% aqueous EtOH extract of the air-dried stems of *C. kaichianum* was suspended in H_2O, and then partitioned with petroleum ether (PE), AcOEt, and BuOH successively. The AcOEt-soluble fraction was further chromatographed on silica gel and *Sephadex LH-20*, and preparative HPLC afforded diterpenoids **1**–**6**.

17-Hydroxyteuvincenone G (**1**) was obtained as yellowish needles and assigned the molecular formula $C_{20}H_{24}O_6$ on the basis of HR-ESI-MS (*m/z* 359.1499 ($[M-H]^-$, $C_{20}H_{23}O_6^-$; calc. 359.1493), which indicated nine degrees of unsaturation. The IR spectrum showed the absorption bands for OH (3420 cm^{-1}), ketones (1714, 1678 cm^{-1}), and benzene moieties (3034, 1612, 1580, 1508 cm^{-1}). The absorption bands in the UV spectrum (235, 295, and 380 nm) also indicated the presence of a benzene ring and a ketone.

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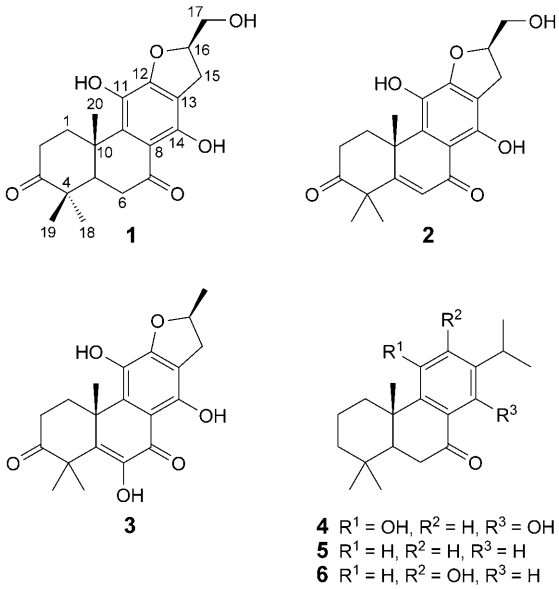

The ^{1}H-NMR spectrum (*Table 1*) of **1** showed signals corresponding to three Me groups (δ(H) 1.17, 1.16, and 1.42 (*s*, 3 H each)) at quaternary C-atoms, which were almost identical to those of teuvincenone G assigned by *Cuadrado et al.* [9] to the Me(18), Me(19) and Me(20) groups (δ(H) 1.18, 1.17 and 1.44, resp.). Signal corresponding to three OH groups (*singlet*s at δ(H) 13.15, 6.30, and 5.39), the strongly deshielded resonance of an OH H-atom (δ(H) 13.15), together with its slow exchange with D_2O, confirmed the existence of a phenolic OH group at C(14), which forms a strong intramolecular H-bond with the C(7)=O function.

In total, 20 C-atom signals were observed in the ^{13}C-NMR and DEPT 135 spectra of **1** (*Table 1*), including two C=O signals at δ(C) 216.0 and 203.1, and six aromatic C-atom signals at δ(C) 155.5, 155.3, 137.3, 131.4, 111.1, and 110.2. The high-field region displayed three Me signals at δ(C) 17.5, 20.9, and 27.0, five CH_2 signals at δ(C) 28.7, 34.4, 35.3, 35.7, and 64.7, two CH signals at δ(C) 49.1 and 86.6, and two signals at (δ(C) 39.3 and 46.9) for quaternary C-atoms (*Table 1*). These data revealed that compound **1** was a diterpenoid. The NMR spectra of **1**, being very similar to those of teuvincenone G [8], which showed the presence of a dihydro-2-(hydroxymethyl)furan fused with the aromatic ring (δ(H) 3.35 (*dd*, J = 15.0, 9.0, H_α–C(15)), 3.31 (*dd*, J = 15.3, 7.0, H_β–C(15)); 5.13 – 5.17 (*m*, H–C(16)); 3.90 and 3.80 (2*dd*, H_α–C(17) and H_β–C(17)); δ(C) 28.7 (*t*, C(15)); 86.6 (*d*, C(16)); 64.7 (*t*, C(17))) instead of the dihydro-2-methylfuran moiety in teuvincenone G (δ(H) 3.38 and 2.85 (2*dd*, J_{gem} = 15.3, H_α–C(15) and H_β–C(15)); 5.14 (*m*, H–C(16)); 1.52 (*d*, Me(17)); δ(C) 34.3 (*t*, C(15)); 83.5 (*d*, C(16)); 22.0 (*q*, C(17))).

Based on the analyses of the ^{1}H,^{1}H-COSY and HSQC data, a *ABX* system (δ(H) 2.41 (*dd*); 2.60 – 2.63 (*m*); 2.60 – 2.63 (*m*)) due to the position 5 and 6 of ring *B* was deduced, indicating the presence of the following fragments: CH_2(1)–CH_2(2),

Table 1. *^{13}C-* (125 MHz) *and ^{1}H-NMR* (500 MHz) *Data*[a]) *of Compound* **1**. Recorded in $CDCl_3$, δ in ppm, J in Hz.

	δ(C)	δ(H)	HMBC (H → C)
H_α–C(1)	35.7 (*t*)	1.99 (*ddd*, *J* = 13.7, 9.7, 9.7)	C(2), C(3), C(5), C(20)
H_β–C(1)		3.28 (*dd*, *J* = 13.7, 7.0)	
H_α–C(2)	34.4 (*t*)	2.73 (*dd*, *J* = 14.5, 7.9)	C(1), C(3), C(4)
H_β–C(2)		2.60–2.63 (*m*)	
C(3)	216.0 (*s*)		
C(4)	46.9 (*s*)		
H–C(5)	49.1 (*d*)	2.41 (*dd*, *J* = 11.2, 7.5)	C(1), C(4), C(6), C(7), C(10)
H_α–C(6)	35.3 (*t*)	2.60–2.63 (*m*)	C(5), C(7), C(10)
H_β–C(6)		2.60–2.63 (*m*)	
C(7)	203.1 (*s*)		
C(8)	110.2 (*s*)		
C(9)	137.3 (*s*)		
C(10)	39.3 (*s*)		
C(11)	131.4 (*s*)		
C(12)	155.3 (*s*)		
C(13)	111.1 (*s*)		
C(14)	155.5 (*s*)		
H_α–C(15)	28.7 (*t*)	3.35 (*dd*, *J* = 15.3, 9.0)	C(12), C(13), C(14), C(16)
H_β–C(15)		3.31 (*dd*, *J* = 15.3, 7.0)	
H–C(16)	86.6 (*d*)	5.13–5.17 (*m*)	C(13), C(15), C(17)
H_α–C(17)	64.7 (*t*)	3.90 (*dd*, *J* = 15.0, 8.7)	C(15), C(16)
H_β–C(17)		3.80 (*dd*, *J* = 15.0, 7.2)	
Me(18)	27.0 (*q*)	1.17 (*s*)	C(3), C(4), C(5), C(19)
Me(19)	20.9 (*q*)	1.16 (*s*)	C(3), C(5), C(18)
Me(20)	17.5 (*q*)	1.42 (*s*)	C(1), C(5), C(9), C(10)
11-OH		5.39 (br. *s*)	
14-OH		13.15 (br. *s*)	

[a]) Assignments were accomplished by a combination of 1D- and 2D- (^1H,^1H-COSY, HSQC, and HMBC) NMR experiments.

CH(5)–CH_2(6), and CH_2(15)–CH(16)–CH_2(17) should exist. In the HMBC spectrum, the ^1H,^{13}C long-range correlations H–C(1)/C(2), C(3), C(5), C(10), and C(20); H–C(5)/C(1), C(3), C(4), C(6), C(7), and C(10); H–C(17)/C(15), and C(16); and H–C(15)/C(13), C(14), C(15), C(16), and C(17) (*Table 1*) suggested the abietane diterpenoid framework in the molecule. Furthermore, the ^1H,^{13}C long-range correlations H–C(18)/C(3), C(4), C(5), and C(19); H–C(19)/C(3), C(4), C(5), and C(18); and H–C(20)/C(1), C(5), C(9), and C(10) indicated that C(18) and C(19) were at C(4), and C(20) was at C(10). Therefore, compound **1** possesses an abietane-type diterpenoid framework with a CH_2OH group at C(16); the absolute configuration at C(16) was not ascertained. However, on biogenetic basis, it is reasonable to assume that it possess the same absolute configuration as the known abietane diterpenoid **3**, which was isolated from the same plant. Thus, the structure of **1** was suggested to be (16*R*)-12,16-epoxy-11,14,17-trihydroxy-17(15 → 16)-*abeo*-abieta-8,11,13-triene-2,7-dione.

17-Hydroxyteuvincenone G (**2**) was obtained as yellowish needles and assigned the molecular formula $C_{20}H_{22}O_6$ on the basis of HR-ESI-MS (*m/z* 357.1330 ($[M-H]^-$, $C_{20}H_{21}O_6^-$; calc. 357.1336), which indicated ten degrees of unsaturation. The IR spectrum showed the absorption bands for OH (3402 cm^{-1}) and ketone (1719 and 1672 cm^{-1}) groups, and benzene moieties (1612, 1582, and 1510 cm^{-1}). The absorption bands in the UV spectrum (265, 296, 336, and 382 nm) also indicated the presence of a benzene ring.

The ^{13}C-NMR and DEPT-NMR spectra indicated that **2** contained three Me, four CH_2, two CH groups, and eleven quaternary C-atoms. The NMR spectra of **2** were very similar to those of compound **1** except that the signals at δ(C) 49.1 and 35.3 were missing, which were replaced by two signals at δ(C) 174.1 and 122.4, indicating an additional C(5)=C(6) bond (*Table 2*). Thus, the structure of **2** was suggested to be (16*R*)-12,16-epoxy-11,14,17-trihydroxy-17(15 → 16)-*abeo*-abieta-5,8,11,13-tetraene-2,7-dione.

Table 2. *^{13}C-* (125 MHz) *and ^{1}H-NMR* (500 MHz) *Data*[a]) *of Compound* **2**. Recorded in $CDCl_3$, δ in ppm, *J* in Hz.

	δ(C)	δ(H)	HMBC (H → C)
H_α–C(1)	26.6 (*t*)	1.96–2.01 (*m*)	C(2), C(3), C(5), C(20)
H_β–C(1)		3.26 (*dd*, *J* = 14.5, 7.0)	
H_α–C(2)	33.2 (*t*)	2.70–2.73 (*m*)	C(1), C(3), C(4)
H_β–C(2)		2.70–2.73 (*m*)	
C(3)	213.2 (*s*)		
C(4)	49.5 (*s*)		
C(5)	174.2 (*s*)		
H–C(6)	122.4 (*d*)	6.30 (*s*)	C(4), C(5), C(8), C(10)
C(7)	189.5 (*s*)		
C(8)	108.9 (*s*)		
C(9)	135.2 (*s*)		
C(10)	41.6 (*s*)		
C(11)	131.1 (*s*)		
C(12)	154.0 (*s*)		
C(13)	111.7 (*s*)		
C(14)	154.1 (*s*)		
H_α–C(15)	28.8 (*t*)	3.31 (*dd*, *J* = 15.3, 9.0)	C(12), C(13), C(14), C(16)
H_β–C(15)		3.01 (*dd*, *J* = 15.3, 7.0)	
H–C(16)	86.4 (*d*)	5.12–5.16 (*m*)	C(15), C(17)
H_α–C(17)	64.8 (*t*)	3.89 (*dd*, *J* = 15.0, 8.7)	C(15), C(16)
H_β–C(17)		3.80 (*dd*, *J* = 15.0, 7.2)	
Me(18)	29.6 (*q*)	1.40 (*s*)	C(3), C(4), C(5), C(19)
Me(19)	20.1 (*q*)	1.44 (*s*)	C(3), C(4), C(5), C(18)
Me(20)	26.6 (*q*)	1.46 (*s*)	C(1), C(5), C(9), C(10)
11-OH		5.41 (br. *s*)	
14-OH		13.34 (br. *s*)	

[a]) Assignments were accomplished by a combination of 1D- and 2D- (^1H,^1H-COSY, HSQC, and HMBC) NMR experiments.

Biological Studies. Compounds **1** and **2** were evaluated for their cytotoxic activities against the HL-60 and A-549 cell lines *in vitro* by means of the MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) assay [10]. Both compounds were found to be significantly active to inhibit the proliferation of HL-60 and A-549 cell lines with IC_{50} values less than 15 μM, as compared to cisplatin used as positive control (*Table 3*).

Table 3. *Cytotoxicities* (IC_{50} [μM]) *of Compounds* **1** *and* **2** *Isolated from* Clerodendrum kaichianum P. S. HSU

Compound	HL-60	A-549
1	5.95 ± 0.57	9.37 ± 0.24
2	15.91 ± 0.95	10.35 ± 0.51
Cisplatin	4.87 ± 0.89	8.63 ± 1.19

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Experimental Part

General. Column chromatography (CC): silica gel (SiO_2, 200–300 mesh; *Qingdao Marine Chemical Factory*, Qingdao, China) and *Sephadex LH-20* (*Amersham Pharmacia Biotech*, UK). Prep. HPLC: *JAI-9103* (*Japan Analytical Industry*). TLC: *Merck* silica-gel plates; visualization under UV light and by spraying with 10% aq. H_2SO_4, followed by heating. M.p.: *X-4* apparatus; uncorrected. Optical rotations: *Perkin-Elmer 241* automatic polarimeter. UV Spectra: *Shimadzu UV-2550* spectrophotometer, in MeOH; λ_{max} (log ε) in nm. IR Spectra: *Nicolet 380 FT-IR* spectrophotometer (*Thermo Nicolet*); KBr pellets; in cm^{-1}. ^{1}H- and ^{13}C-NMR: *Bruker AVANCE III 500* spectrometer, at 500 (^{1}H), and 125 (^{13}C) MHz; in $CDCl_3$; δ in ppm, *J* in Hz. HR-ESI-MS: *Agilent 6210* TOF-MS mass spectrometer.

Plant Material. The stems of *C. kaichianum* were collected on the mountains of Lin'an County, Zhejiang Province, P. R. China, in September of 2009, and identified by Dr. *Bin Wu* (College of Pharmaceutical Sciences, Zhejiang University, Hangzhou, China). A voucher specimen (No. 20090913) was deposited with the laboratory of Zhejiang Gongshang University, Hangzhou, P. R. China.

Extraction and Isolation. The air-dried powder of the stems (11.6 kg) of *C. kaichianum* was extracted with 75% aq. EtOH three times at 75° for 4 h each. The EtOH extracts were combined and evaporated to dryness to afford a gummy residue (325 g), which was suspended in H_2O (2 l) and extracted at r.t. with petroleum ether (PE; 3 × 2 l, 65 g), AcOEt (3 × 2 l, 102 g), and BuOH (3 × 2 l, 88 g), successively.

Part of AcOEt extract (90 g) was subjected to CC (SiO_2; AcOEt/PE gradient) to afford ten fractions, *Frs. 1–10*, on the basis of TLC analysis. *Fr. 2* (8 g) was further subjected CC (SiO_2; AcOEt/PE 5–15%) and separated into five fraction pools, *Frs. 2A*, *2B*, *2C*, *2D*, *2E*. *Fr. 2D* was further purified on *Sephadex LH-20* and later prep. HPLC (H_2O/MeOH 60:40) to afford compounds **3** (12 mg), **4** (40 mg), **5** (17 mg), and **6** (9 mg).

The major fraction, *Fr. 4* (9 g), was further subjected CC (SiO_2; PE/acetone from 5:1 to 1:1) to give six fraction pools, *Frs. 4A*, *4B*, *4C*, *4D*, *4E*, *4F*. *Fr. 4E* was subjected to *Sephadex LH-20*, and later prep. HPLC to give compounds **1** (8 mg) and **2** (11 mg).

17-Hydroxyteuvincenone G (=(*16*R)*-12,16-Epoxy-11,14,17-trihydroxy-17(15 → 16)*-abeo-*abieta-8,11,13-triene-2,7-dione*; **1**). Yellowish needles. M.p. 250–252°. $[\alpha]_{25}^{D} = +150.1$ ($c = 0.02$, MeOH); UV(MeOH): 207, 225, 280, 360. IR: 3400, 2992, 1717, 1650, 1615, 1699, 1581. ^{1}H- and ^{13}C-NMR ($CDCl_3$): see *Table 1*. HR-ESI-MS: 359.1487 ($[M-H]^-$, $C_{20}H_{23}O_6^-$; calc. 359.1494).

17-Hydroxyteuvincen-5(6)-enone G (=(*16*R)*-12,16-Epoxy-11,14,17-trihydroxy-17(15 → 16)*-abeo-*abieta-5,8,11,13-tetraene-2,7-dione*; **2**). Yellowish needles. M.p. 305–307°. $[\alpha]_{D}^{25} = +47.2$ ($c = 0.07$,

MeOH). UV(MeOH): 203, 260, 299, 365. IR: 3396, 2980, 1716, 1650, 1622, 1693, 1679. ^{1}H- and ^{13}C-NMR ($CDCl_3$): see *Table 2*. HR-ESI-MS: 357.1330 ($[M-H]^-$, $C_{20}H_{21}O_6^-$; calc. 357.1336).

The percentage of growth inhibition was determined using a MTT assay to measure viable cells with minor modification. A total of 5000–10000 exponential phase cells per well was seeded onto a 96-well plate for 24 h, treated with compounds **1** and **2** at 2.5, 5, 10, 20, 40 μM concentrations for 72 h using cisplatin as a positive control. Briefly, MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide; 100 μl) working soln. (1 mg/ml) was added into each well of 96-well plate and incubated at 37° for 4 h, and then the medium was removed. The converted dye formazan was solubilized with 150 μl acidic i-PrOH, and each concentration was tested in triplicate. The absorbance was then measured at a wavelength of 570 nm using a microplate reader. The dose resulting in 50% inhibition of cell growth, IC_{50}, was calculated by NDST software.

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